

**SUPPLEMENTARY INFORMATION FOR:**

**Shotgun Proteomic Analysis Unveils Survival  
and Detoxification Strategies by *Caulobacter  
crescentus* during Exposure to Uranium,  
Chromium, and Cadmium**

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**Table S1. Primers used in qRT-PCR reactions.**

Genes	Primer sequences	Efficiency
<i>cc_1293</i>	Forward 5' CGAACGTGGTCGATGTCTATG 3'	97%
	Reverse 5' CCCTCACCGTGGTGATAAAG 3'	
<i>cc_3145</i>	Forward 5' TCGTCTTCATCTGCGTTTCC 3'	97%
	Reverse 5' GTGGATCTGTTGGCTGTGAT 3'	
<i>cc_1295</i>	Forward 5' TGGATCAATGGCTGCTCTAC 3'	105%
	Reverse 5' ACTTTGCTTCAGACGACAGAT 3'	
<i>cc_0008</i>	Forward 5' TCACCTTCGAGACCTTCGT 3'	115%
	Reverse 5' GTGGAACAGCACAGGATTGA 3'	
<i>cc_3035</i>	Forward 5' CGTACTGTTGATCGAGGATGAC 3'	101%
	Reverse 5' TCACCCAGATCCGTCGTATAG 3'	
<i>cc_0139</i>	Forward 5' GACAGCCAGGTGACCTATTC 3'	110%
	Reverse 5' CGCGCTTGATCTTGAAATGG 3'	
<i>cc_0286</i>	Forward 5' CGGCTTCCCAACAATCAAC 3'	105%
	Reverse 5' TCCAGTCCCGGATCTTCTT 3'	
<i>cc_1598</i>	Forward 5' GGTTCGTCTTCCAGCAGTAT 3'	115%
	Reverse 5' TTCGACGGCTTGTCTTG 3'	
<i>cc_3047 (rpoD)</i>	Forward 5' CTCTATGCGATCAACAAGCG 3'	105%
	Reverse 5' ATAGGCCTTGAGGAACTCGC 3'	

**Table S2. Excel file containing all identified proteins and differentially expressed proteins under each metal condition (submitted separately).****Table S3. Changes in doubling time of *C. crescentus* grown in M2G medium supplemented with 200 or 500  $\mu$ M uranyl nitrate (U200 or U500), 10 or 15  $\mu$ M potassium chromate (Cr10 or Cr15), or 7.5  $\mu$ M cadmium sulfate (Cd), compared to the control with no metal added.**

	Control	U200	U500	Cr10	Cr15	Cd
Doubling time (h)	2.70	2.81	2.77	2.96	3.03	2.90
Change relative to control (%)	0.00	2.59	0.37	9.63	12.2	7.41

**Table S4. Distribution of proteins identified in response to metal exposure.** DE, differentially expressed; Up, up-regulated; Down, down-regulated; U200 and U500, uranyl nitrate at 200  $\mu$ M and 500  $\mu$ M respectively; Cr10 and Cr15, potassium chromate at 10  $\mu$ M and 15  $\mu$ M respectively; Cd, cadmium sulfate at 7.5  $\mu$ M; control, no metal control.

	U200	U500	Cr10	Cr15	Cd	control
Total	947	964	970	971	969	982
DE	101	126	166	147	144	-
Up	27	48	65	78	66	-
Down	74	78	101	69	78	-

**Table S5. Comparison of up-regulated proteins found in the current proteomic study with previous transcriptomic results.**<sup>1</sup> The two numbers under U and Cr denote the number of proteins under each of the two concentrations tested.

	U	Cr	Cd
Proteome	27, 48	65, 78	66
Transcriptome	52	282	150
Common	12	14	21

**Table S6. Proteins differentially expressed under Cr and U exposure conditions.** Relevant promoter motifs<sup>2</sup> are noted in parenthesis adjacent to the corresponding protein names.

Genes	Log2 fold change				Annotation
	Cr10	Cr15	U200	U500	
<b>Up-regulated</b>					
Possible extracellular activities					
CC_1295 (cc_5)	2.6	3.2	6.1 <sup>a</sup>	6.1 <sup>a</sup>	3-phytase/6-phytase
<b>Down-regulated</b>					
Cell cycle					
CC_1485 (cc_11)	-3.1	< -5	-1.0 <sup>b</sup>	-2.9	Small CtrA inhibitory protein
Lipid metabolism					
CC_0430	-2.7 <sup>b</sup>	-2.7 <sup>b</sup>	< -5	< -5	PhbC, poly(3-hydroxyalkanoate) polymerase
Nitrogen metabolism					
CC_0365	< -5	< -5	-1.9	-1.9	Nitropropane dioxygenase/trans-enoyl-CoA reductase family protein

<sup>a</sup> Also transcriptionally differentially expressed based on Hu et al.<sup>1</sup>

<sup>b</sup> 0.05 > FDR > 0.005, all other FDR < 0.005.

**Table S7. Proteins differentially expressed under 500  $\mu$ M uranyl nitrate but not 200  $\mu$ M.**Relevant promoter motifs<sup>2</sup> are noted in parenthesis adjacent to the corresponding protein names.

Genes	Log2 fold change	Annotation
<b>Up-regulated</b>		
Stress response		
CC_1178	>5	Conserved putative cytosolic general stress protein, GsiB-like
CC_2258	1.2	Small heat shock protein
Outer membrane functions		
CC_0815 (cc_1)	1.2	TonB-dependent receptor, CirA-like for Fe transport
CC_3373	>5	ABC-type multidrug transport system, ATPase component
Cell wall biosynthesis		
CC_2033	>5	Aspartate racemase
Unknown		
CC_0846	2.1	Conserved hypothetical protein, putative lumazine binding
CC_1037	1.8	Conserved hypothetical protein
CC_1532	2.2	Conserved hypothetical protein
<b>Down-regulated</b>		
Cell motility/chemotaxis		
CC_0432	-1.0	CheYI, chemotaxis receiver domain protein
CC_0433	-1.5	CheAI, chemotaxis histidine kinase protein
CC_0441	< -5	CheE protein
Pilus/host cell attachment		
CC_2519	-1.8	Host cell attachment protein
CC_2944	-2.6	CpaD, pilus assembly protein
Others		
CC_0116	-1.5	TolB protein, putative aminohydrolase
CC_1041	< -5	Hypothetical protein

**Table S8. Proteins differentially expressed under 15  $\mu$ M potassium chromate but not 10  $\mu$ M.**

Genes	Log2 fold change	Annotation
<b>Up-regulated</b>		
Protein folding/heat shock response		
CC_0011	1.3	DnaJ, chaperone protein
Fe-S cluster biosynthesis		
CC_2009	>5	HesB/YadR/YfhF family protein
Organic solvent resistance		
CC_3693	>5 <sup>a</sup>	Tig2D, organic solvent resistance ABC transport system protein
CC_3694	3.2	Tig2C, organic solvent resistance ABC transport system protein
Translation inhibition		
CC_1089	>5	Translation initiation inhibitor
Amino acid biosynthesis		
CC_3606	1.0	GltD, glutamate synthase (NADPH) small chain
Unknown		
CC_0600	1.1	Hypothetical protein
CC_1245	1.8	Hypothetical protein
CC_1550	>5	Conserved hypothetical protein, LabA-like
CC_3495	>5	SAM-dependent methyltransferase
<b>Down-regulated</b>		
Cell motility/chemotaxis		
CC_0431	< -5	CheX protein
Transcription/translation		
CC_0769	-2.1	RpmH, ribosomal protein L34
CC_1247	-1.2	RpsJ, ribosomal protein S10
CC_0986	< -5	Transcriptional regulator, GntR family, FadR-like
Outer membrane functions		
CC_0991	-1.1	TonB-dependent receptor, CirA-like for Fe transport
Glutathione metabolism		
CC_3088	< -5	Glutathione S-transferase
Unknown		
CC_1031	< -5	Hypothetical protein

<sup>a</sup> 0.05 > FDR > 0.005, all other FDR < 0.005.

**Table S9. Proteins differentially expressed under Cd and U exposure conditions.** Relevant promoter motifs<sup>2</sup> are noted in parenthesis adjacent to the corresponding protein names.

Genes	Log2 fold change			Annotation
	Cd	U200	U500	
<b>Up-regulated</b>				
Cell wall biosynthesis				
CC_1913	>5	>5	>5	FirA, UDP-3-O-acylglucosamine N-acyltransferase
<b>Down-regulated</b>				
Cell cycle				
CC_1485	< -5	< -5	-3.0	TipN, polar localization protein
CC_3226	-2.3	-2.2	-2.2	FtsH, ATP-dependent zinc metalloprotease, cell division
Cell motility/chemotaxis				
CC_0430 (cc_10)	-1.4	-2.3	-2.3	McpA, chemoreceptor
Oxidative phosphorylation				
CC_0365	-1.7	-1.8	-1.1	AtpF, ATP synthase subunit b
CC_0366	-1.5	-1.4	-1.1	AtpF, ATP synthase subunit b2
Cell wall biosynthesis				
CC_0164	-2.2	-1.7	-1.6	Chain length regulator/tyrosine-protein kinase, capsular polysaccharide biosynthesis
CC_0169	< -5	< -5	< -5	Putative capsule polysaccharide export protein
Outer membrane function				
CC_0214 (cc_13)	-1.4 <sup>a</sup>	-1.3	-1.2	TonB-dependent receptor, CirA-like for Fe transport
CC_1517 (cc_13)	-1.3	-1.5	-1.3	TonB-dependent receptor, siderophore transport
CC_1518	-3.4 <sup>a</sup>	-3.3	-3.7	ABC-type multidrug transport system, ATPase component
CC_2194 (cc_13)	-1.3	-1.3	-1.7	TonB-dependent hemin receptor
CC_2336	-1.8	-1.7	-1.8	ExbB, MotA/TolQ/ExbB proton channel family protein, biopolymer transport
Unknown				
CC_0075	< -5	< -5	< -5	Conserved HemY domain membrane protein

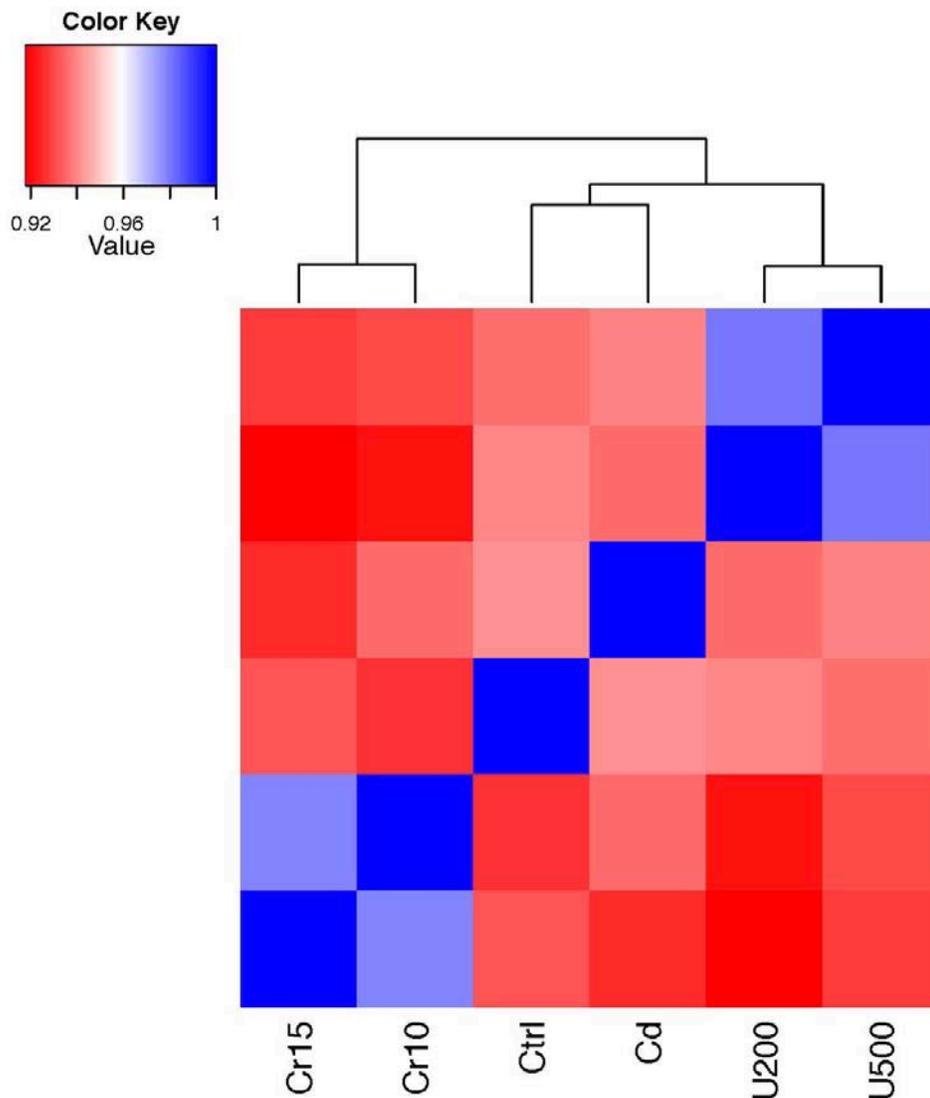
<sup>a</sup> Also transcriptionally differentially expressed based on Hu et al.<sup>1</sup>

**Table S10. Proteins differentially expressed under Cd and Cr exposure conditions.** Relevant promoter motifs<sup>2</sup> are noted in parenthesis adjacent to the corresponding protein names.

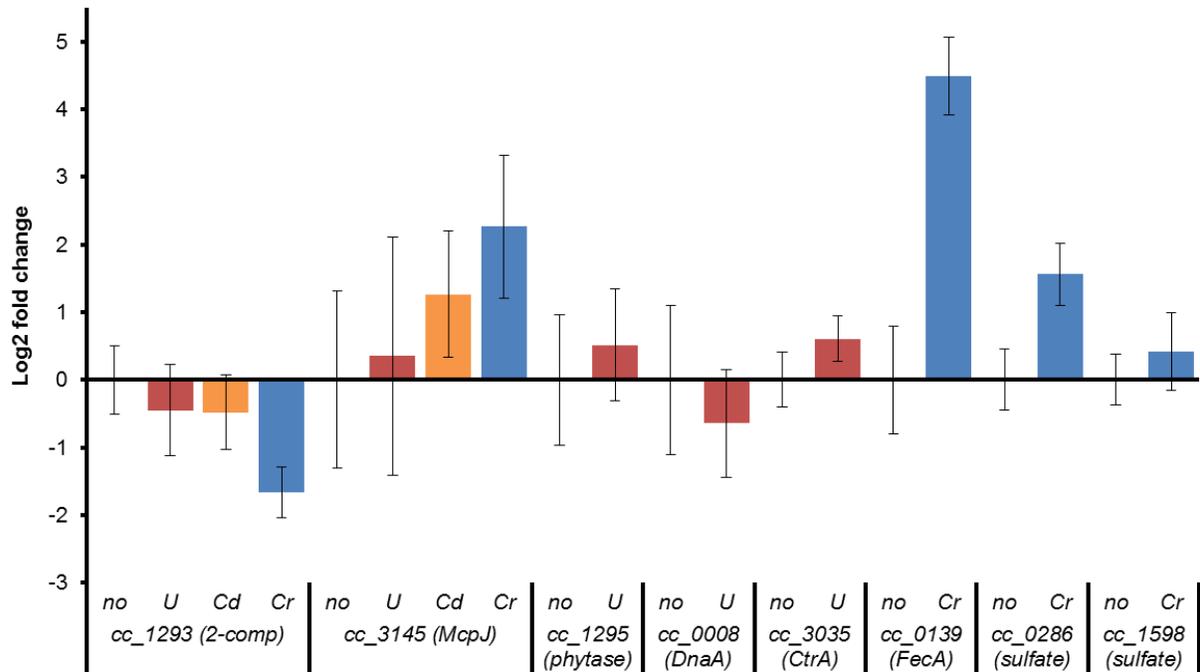
Genes	Log2 fold change			Annotation
	Cd	Cr10	Cr15	
<b>Up-regulated</b>				
<b>Protein folding response</b>				
CC_2799	1.1	1.6	1.9	Fk1B, peptidyl-prolyl cis-trans isomerase, FKBP-type
CC_3504	>5 <sup>a</sup>	>5 <sup>b</sup>	>5 <sup>b</sup>	Peptidase M13 family protein, PepO-like
<b>Outer membrane functions</b>				
CC_3502	1.2 <sup>a</sup>	1.7 <sup>b</sup>	1.4 <sup>a,b</sup>	Putative exported protein, predicted Cu(I) binding
CC_3373 (m_6)	>5	>5	>5	ABC-type multidrug transport system, ATPase component
<b>Potential oxidative stress</b>				
CC_2653 (m_6)	2.2 <sup>b</sup>	1.3 <sup>a</sup>	2.2	Nitroreductase family protein
<b>Unknown</b>				
CC_0846	2.2	3.3	3.4	Hypothetical protein, putative lumazine-binding
CC_3107	>5	>5	>5	Conserved aminopeptidase, M20/M25/M40 family
<b>Down-regulated</b>				
<b>Redox balance</b>				
CC_0375	-2.1	-1.8	-1.1	DsbA, thiol:disulfide interchange protein
CC_1210	< -5	< -5	< -5	Cytochrome c family protein
<b>Outer membrane functions</b>				
CC_2320	< -5	< -5	< -5	ABC-type transport system, periplasmic component

<sup>a</sup> 0.05 > FDR > 0.005, all other FDR < 0.005.

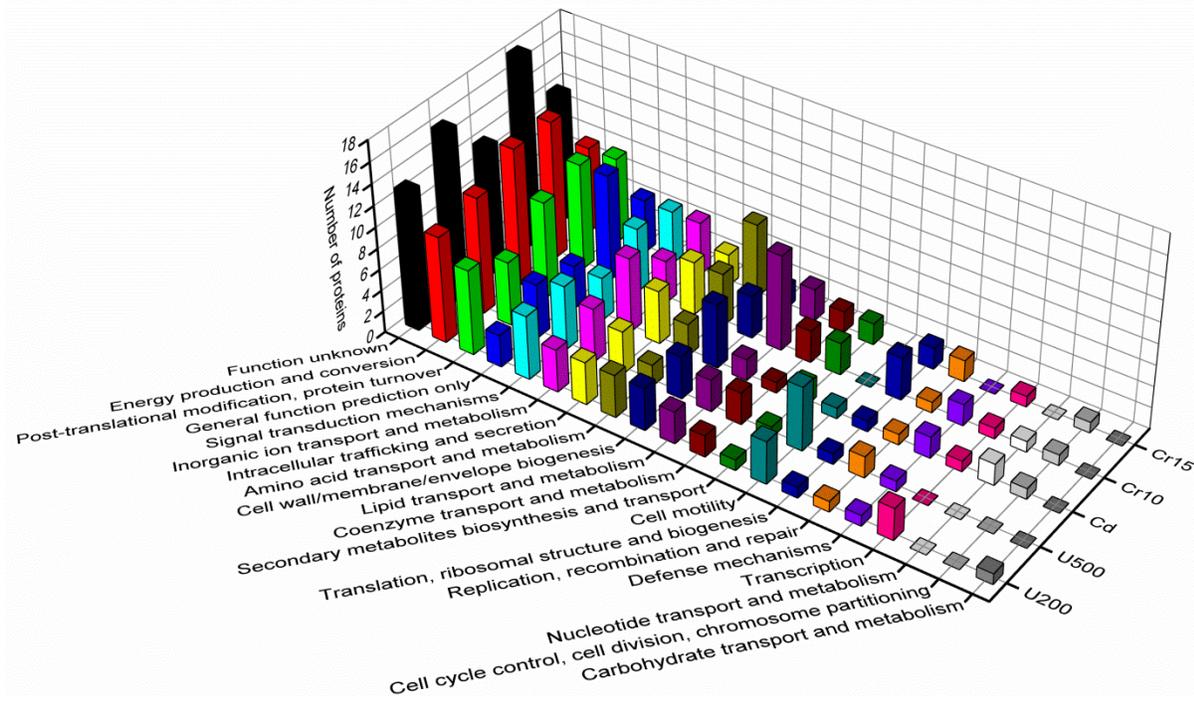
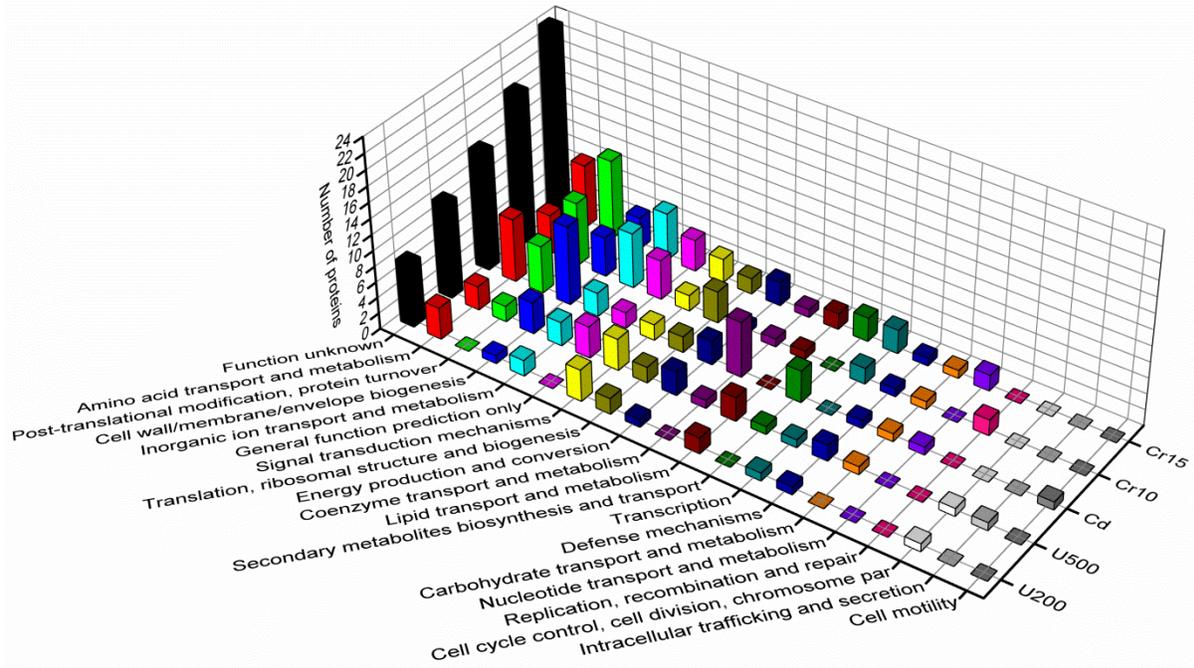
<sup>b</sup> Also transcriptionally differentially expressed based on Hu et al.<sup>1</sup>



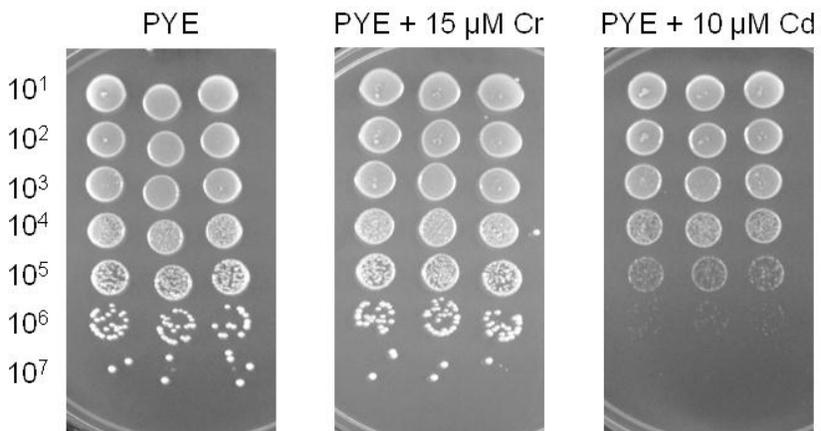
**Figure S1. Cluster analysis of proteomic profiles under all conditions based on Spearman rank correlation.** Total spectra counts were averaged over the triplicate after mean scaling normalization. For a protein to be included, it required at least 20 total spectral counts across the 6 conditions. A total of 809 proteins were included. U200, uranyl nitrate at 200  $\mu\text{M}$ ; U500, uranyl nitrate at 500  $\mu\text{M}$ ; Cr10, potassium chromate at 10  $\mu\text{M}$ ; Cr15, potassium chromate at 15  $\mu\text{M}$ ; Cd, cadmium sulfate at 7.5  $\mu\text{M}$ .



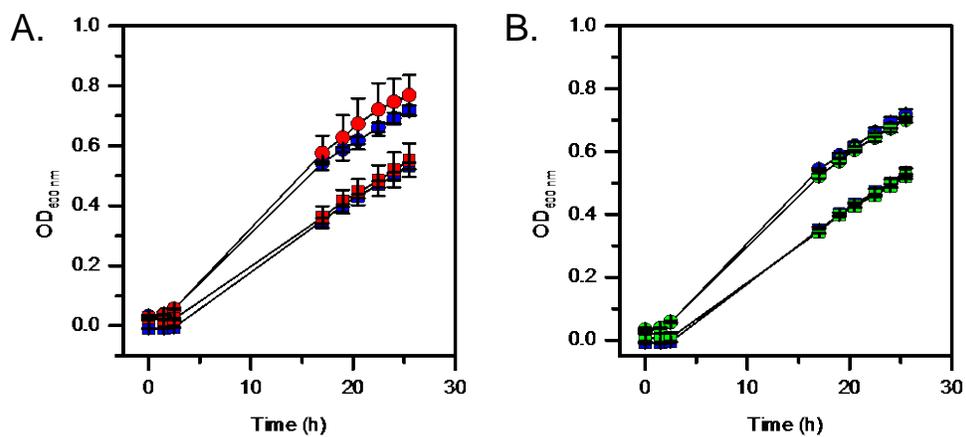
**Figure S2. RNA expression levels of select proteins based on qRT-PCR.** RNA was extracted from cells treated with no metal (no), 200  $\mu$ M uranyl nitrate (U, red), 7.5  $\mu$ M cadmium sulfate (Cd, orange), or 15  $\mu$ M potassium chromate (Cr, blue). Relative changes in expression levels compared to the no metal control are shown. All samples were normalized to the housekeeping gene *rpoD*. Error bars denote error from biological triplicates.



**Figure S3. COG distribution of differentially expressed proteins.** COGs were assigned according to Hottes et al.<sup>3</sup> Top, up-regulated proteins; Bottom, down-regulated proteins. U200, uranyl nitrate at 200  $\mu\text{M}$ ; U500, uranyl nitrate at 500  $\mu\text{M}$ ; Cr10, potassium chromate at 10  $\mu\text{M}$ ; Cr15, potassium chromate at 15  $\mu\text{M}$ ; Cd, cadmium sulfate at 7.5  $\mu\text{M}$ .



**Figure S4. Growth of serial dilutions of *C. crescentus* CB15N on PYE-agar (1.5% w/v) supplemented with Cd or Cr. Pictures of the plates after two-day incubation at 30 °C.**



**Figure S5. Growth of CC<sub>1293</sub>/CC<sub>1304</sub> double mutant (A) and CC<sub>2091</sub> ABC transporter mutant (B) in PYE medium supplemented with uranium.** Wild-type, blue; CC<sub>1293</sub>/CC<sub>1304</sub> mutant, red; CC<sub>2091</sub> mutant, green. PYE supplemented with 300  $\mu$ M uranyl nitrate, squares; PYE with no metal, circles. Error bars denote standard deviation from triplicate cultures.

## REFERENCES:

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2. McGrath, P. T.; Lee, H.; Zhang, L.; Iniesta, A. A.; Hottes, A. K.; Tan, M. H.; Hillson, N. J.; Hu, P.; Shapiro, L.; McAdams, H. H. High-throughput identification of transcription start sites, conserved promoter motifs and predicted regulons. *Nat. Biotechnol.* **2007**, *25*, 584-592.
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