Supporting Information

Title

Cobalt release from a nanoscale multiphase lithiated cobalt phosphate dominates interaction with *Shewanella oneidensis* MR-1 and *Bacillus subtilis* SB491

Authors

Peter L. Clement,[†] Joshua E. Kuether,[‡] Jaya R. Borgatta,[§] Joseph T. Buchman,[†] Meghan S. Cahill,[†] Tian A. Qiu,[†] Robert J. Hamers,[§] Z. Vivian Feng,[‡] Christy L. Haynes[*†]

Addresses

†University of Minnesota, Department of Chemistry, 207 Pleasant St. SE, Minneapolis, Minnesota 55455, United States
‡Augsburg University, Department of Chemistry, 2211 Riverside Ave., Minneapolis, Minnesota 55454, United States
§Department of Chemistry, University of Wisconsin, 1101 University Avenue, Madison, Wisconsin 53706, United States

Reagents

NaCl (Sigma-Aldrich), HEPES (Sigma-Aldrich), BD™ Difco™ Dehydrated Luria-Bertani (LB) broth, Dulbecco's phosphate-buffered saline without Ca and Mg (Corning), cobalt chloride hexahydrate (Mallinckrodt), 30% H_2O_2 (Themo Fisher Scientific), 3′-*p*-aminophenyl fluorescein (APF), dichlorofluorescin diacetate (H_2DCFDA), Newport Green™ DCF-DA (Invitrogen, Thermo Fisher Scientific), HRP (Cayman Chemicals), and NaOH (Macron) were purchased and used without further purification.

Table S1. Full abiotic fluorescence responses of H_2DCF, APF, and Newport Green™ DCF to mLCP and controls (ex./em. 505/535 nm).

	APF		H_2DCF		Newport Green™ DCF	
Negative control	30 ± 5[a]		170 ± 50[b]		400 ± 20[a]	
$CoCl_2$	30 ± 20[a]		330 ± 60[a]		1200 ± 100[a]	
mLCP	30 ± 10[a]		600 ± 500[b]		1370 ± 60[a]	
H_2O_2	30 ± 5[a]		200 ± 20[a]		440 ± 50[a]	
HRP	210 ± 10[c]	2400 ± 300[d]	12000 ± 200[c]	39300 ± 500[d]	700 ± 40[c]	400 ± 20[d]
Pos.	1850 ± 8[c]	3100 ± 200[d]	33400 ± 600[c]	38600 ± 300[d]	1250 ± 20[c]	2130 ± 60[d]
Pos. + mLCP	2070 ± 30[c]	1800 ± 90[d]	32000 ± 2000[c]	38400 ± 500[d]	130 ± 30[c]	1500 ± 90[d]

a. Entries are means ± standard deviations from two material replicates

b. Entries are means ± standard deviations from three material replicates

c. Entries are means ± standard deviations from one material replicates (three analytical replicates). HRP concentration is 0.2 units/mL.

d. Entries are means ± standard deviations from one material replicates (three analytical replicates). HRP concentration is 2 units/mL.

Table S2. Visual MINTEQ simulation of ionic speciation assuming release of 100 micromolal Co^{2+}. Predominant cobalt species are bolded for clarity.

Co^{2+}/Co^{3+} redox disabled					Co^{2+}/Co^{3+} redox enabled			
	Concentration (Molal)					Concentration		
	100 micromolal Co^{2+}	50 micromolal Co^{2+}, 50 micromolal Co^{3+},	100 micromolal Co^{3+}			100 micromolal Co^{2+}	50 micromolal Co^{2+}, 50 micromolal Co^{3+},	100 micromolal Co^{3+}
Cl-1	0.019851	0.019851	0.019851		Cl^{-1}	0.019851	0.019851	0.019851
$Co(OH)_2$ (aq)	5.7062E-09	2.85E-09			$Co(OH)_2$ (aq)	5.7062E-09	5.71E-09	5.71E-09
$Co(OH)_3^-$	3.326E-14	1.66E-14			$Co(OH)_3^-$	3.326E-14	3.33E-14	3.33E-14
Co^{+2}	**0.00009916**	**4.96E-05**			Co^{+2}	**0.00009916**	**9.92E-05**	**9.92E-05**
Co^{+3}		7.84E-11	1.57E-10		Co^{+3}	3.1658E-29	3.17E-29	3.17E-29
$Co_4(OH)_4^{+4}$	1.2477E-17	7.8E-19			$Co_4(OH)_4^{+4}$	1.2477E-17	1.25E-17	1.25E-17
$CoCl^+$	5.0211E-07	2.51E-07			$CoCl^+$	5.0211E-07	5.02E-07	5.02E-07
$CoCl^{+2}$		1.37E-10	2.73E-10		$CoCl^{+2}$	5.518E-29	5.52E-29	5.52E-29
$CoOH^+$		1.64E-07			$CoOH^+$	3.285E-07	3.29E-07	3.29E-07
$CoOH^{+2}$	3.285E-07	**5E-05**	**1E-04**		$CoOH^{+2}$	2.0185E-23	2.02E-23	2.02E-23
H^{+1}	4.5796E-08	4.58E-08	4.58E-08		H^{+1}	4.5796E-08	4.58E-08	4.58E-08
$HEPES^{-1}$	0.0008841	0.000884	0.000884		$HEPES^{-1}$	0.0008841	0.000884	0.000884
H-HEPES (aq)	0.0011159	0.001116	0.001116		H-HEPES (aq)	0.0011159	0.001116	0.001116
Na^{+1}	0.019851	0.019851	0.019851		Na^{+1}	0.019851	0.019851	0.019851
NaCl (aq)	0.00014854	0.000149	0.000149		NaCl (aq)	0.00014854	0.000149	0.000149
NaOH (aq)	5.4651E-09	5.47E-09	5.47E-09		NaOH (aq)	5.4651E-09	5.47E-09	5.47E-09
OH^-	2.9075E-07	2.91E-07	2.91E-07		OH^-	2.9075E-07	2.91E-07	2.91E-07

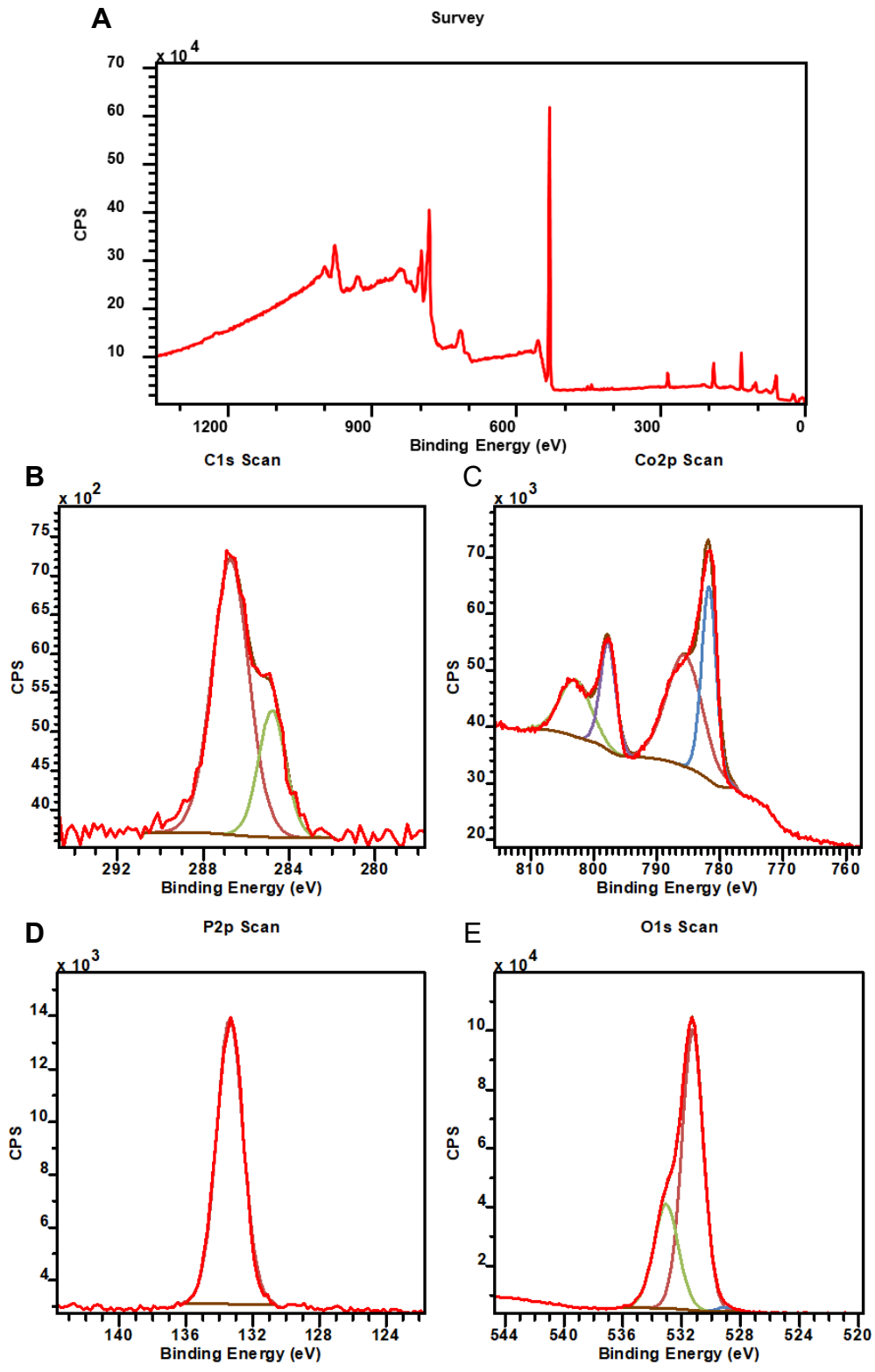


Figure S1. XPS analysis of mLCP including (A) a survey spectrum and a multiplex analysis of (B) carbon, (C) cobalt, (D) phosphorus, and (E) oxygen.

The XPS characterization of the mixed phase lithium cobalt phosphate material gave insight in to the oxidation state of cobalt and phosphorus (shown in Figure S1). The phosphorus and cobalt had peaks consistent with a characterization data for cobalt phosphate.[1] The phosphorus peak at 133.36 eV had a similar position to phosphorus previously reported in cobalt phosphate materials, at 133.7 eV.[1] The cobalt $2p_{3/2}$ and $2p_{1/2}$ peaks were at 781.76 eV and 797.75 eV, respectively, similar peaks were reported at 781.16 eV and 797.08 eV. In addition, the peak separations showed that the cobalt oxidation state was most similar to Co(II); the $2p_{3/2}$ and $2p_{1/2}$ peaks were split by a distance of 15.99 eV which is comparable to the literature Co(II)O peak splitting of 15.9 eV. In addition, the satellite peaks are separated by 3.87 eV and 5.16 eV for $2p_{3/2}$ and $2p_{1/2}$, respectively, which is comparable to literature peak separations of 5.9 eV and 6.7 eV.[2]

For *S. oneidensis*, we validated the GBV assay using a lower-throughput colony counting assay. The colony counting assay (Figure S2) shows higher viabilities than those measured using the GBV for the *S. oneidensis* to mLCP and $CoCl_2$. We note that colonies were notably smaller at the 100 mg/L mLCP and 100 µM $CoCl_2$ doses, therefore, colony counts alone do not fully capture the influence of particles or toxic ions on viable bacteria.

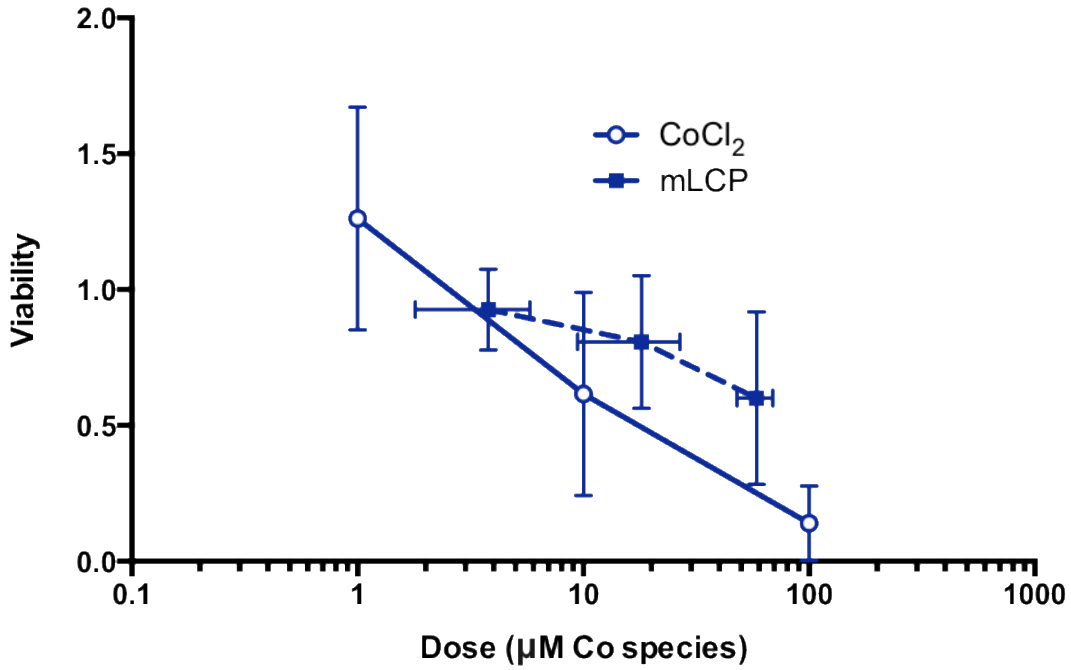


Figure S2. Viability of *S. oneidensis* MR-1 exposed to either mLCP or $CoCl_2$, as determined by colony counting viability assay. Doses are presented based on soluble [Co^{2+}] at 1 hour of particles in HEPES, as determined by ICP for mLCP. Data represent means of triplicates from different days. Error bars represent standard deviations.

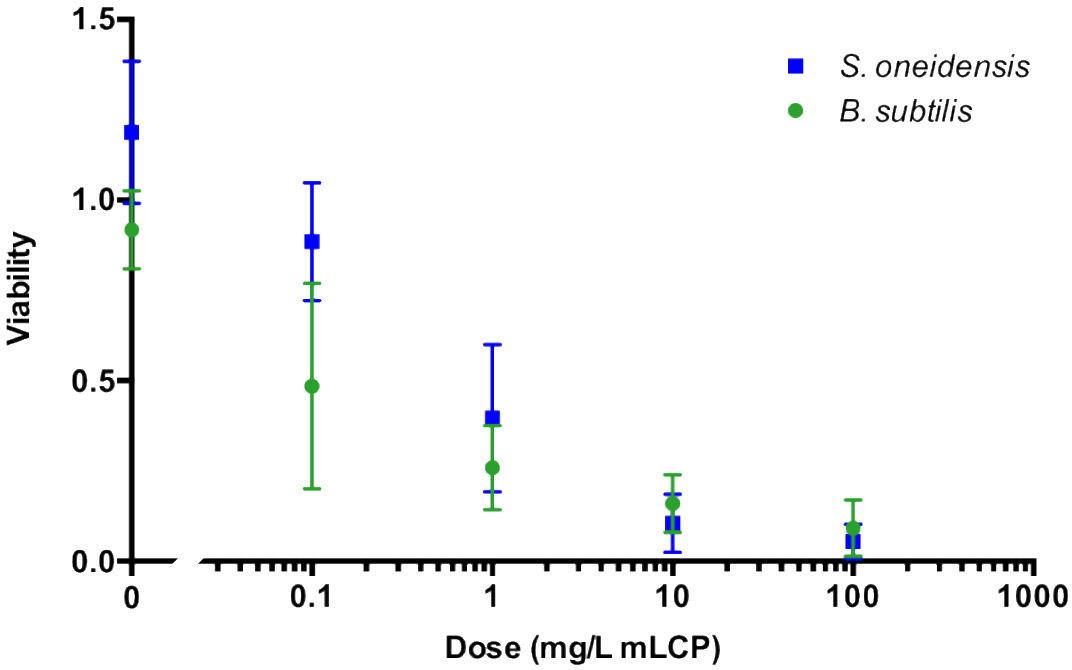


Figure S3. Viability of *S. oneidensis* MR-1 (blue) and *B. subtilis* (green) exposed to mLCP as determined by GBV. Doses presented here are based on mass of particle used in the exposure. Data for *S. oneidensis* MR-1 represent a mean of biological triplicates; data for B. subtilis represent

a mean of four biological replicates. We excluded one biological replicate of *B. subtilis* exposed to both ions and mLCP. Error bars represent standard deviations.

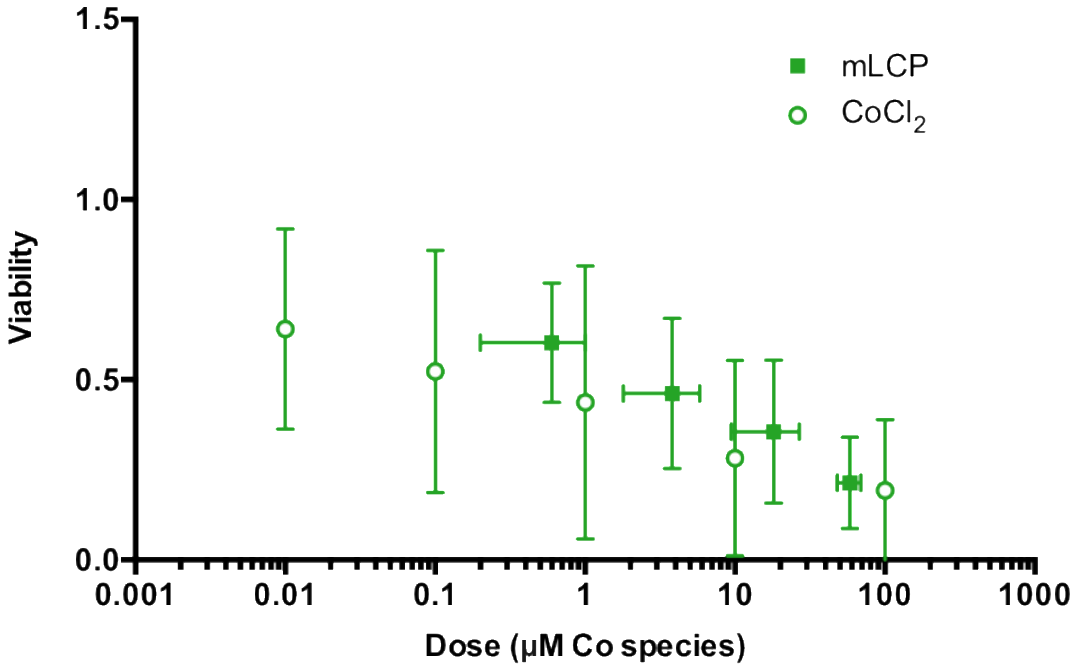


Figure S4. Viability of *B. subtilis* exposed to either mLCP or $CoCl_2$, as determined by GBV. Doses are presented based on soluble [Co^{2+}] at 1 hour of particles in HEPES, as determined by ICP for mLCP. Data for B. subtilis represent means of five replicates from different days. **We did not exclude one biological replicate of *B. subtilis* exposed to both ions and mLCP here. Error bars represent standard deviations.**

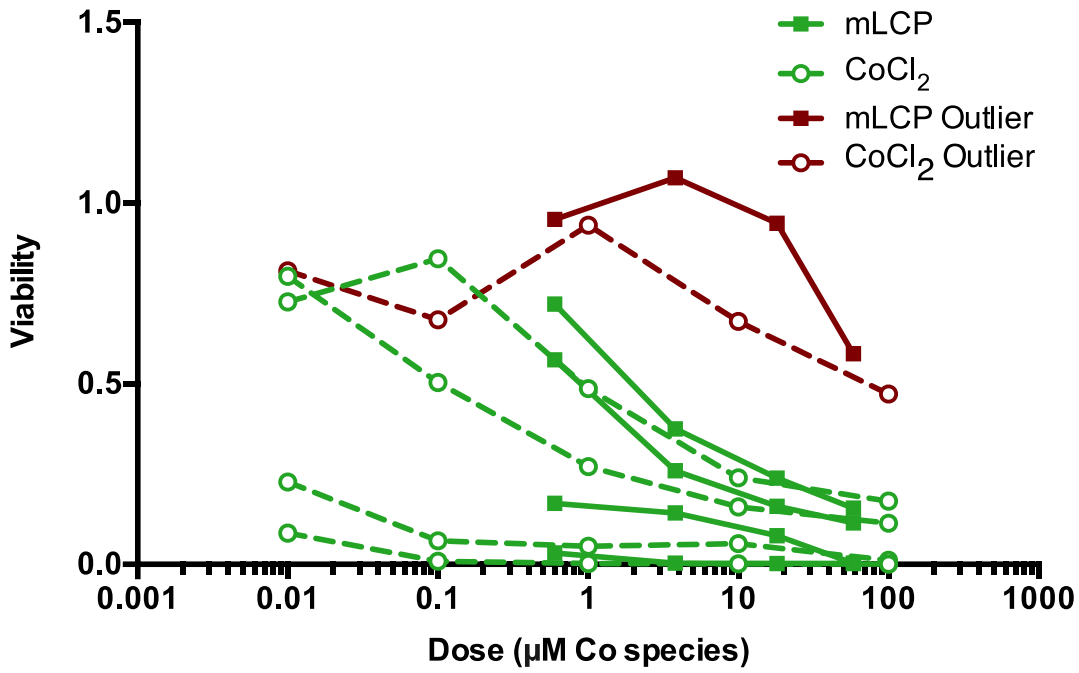
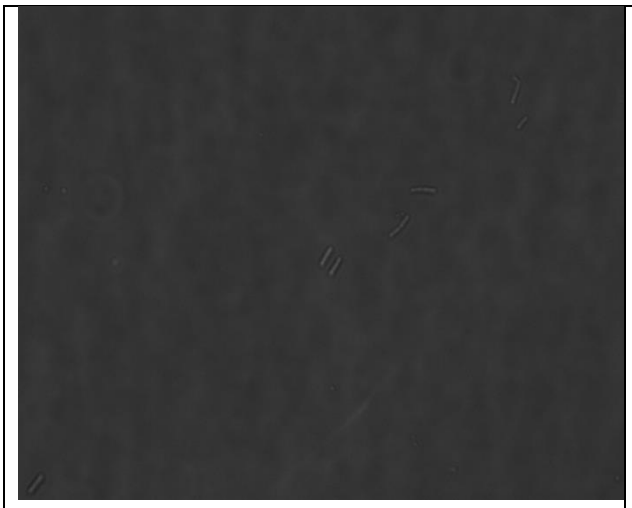
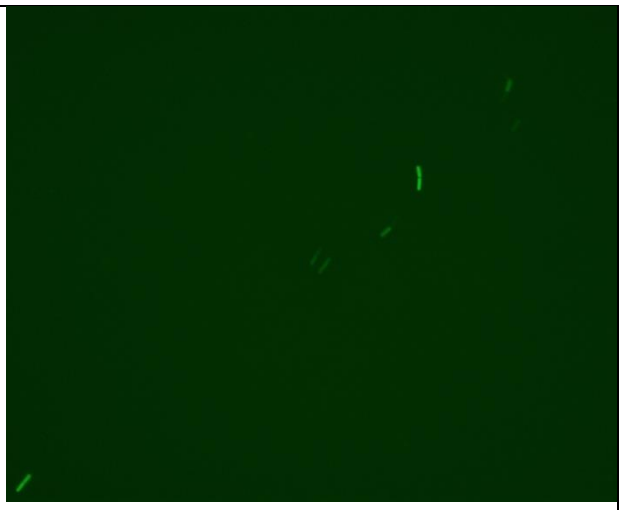


Figure S5. Viability of *B. subtilis* exposed to either mLCP or $CoCl_2$, as determined by GBV. Doses are presented based on soluble [Co] at 1 hour of particles in HEPES, as determined by ICP for mLCP. Data for *B. subtilis* represent means of five replicates from different days. The one abnormal biological replicate is denoted in red, as determined by behavior at high doses. This replicate was excluded for mLCP and $CoCl_2$ based on outlier testing at the two highest doses.

We validated the NPG assay using fluorescence microscopy to ensure the fluorescence signal was originated from dye retained in bacterial cells. In Figures S6 and S7 we show that for both *S. oneidensis* and *B. subtilis* Newport Green™ DCF fluorescence dye is localized to cells. Additionally, we show that there is minimal fluorescence for bacteria loaded with Newport Green™ DCF but not treated with Co^{2+} ions.

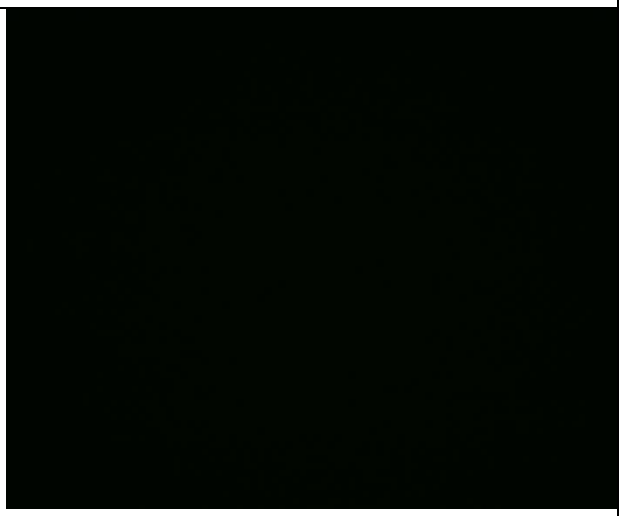


(a)



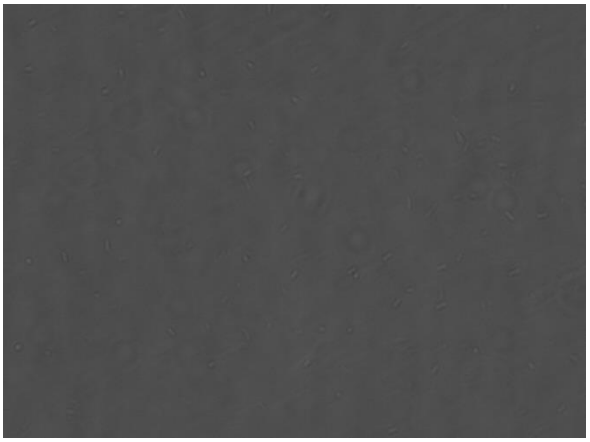
(b)

(c)

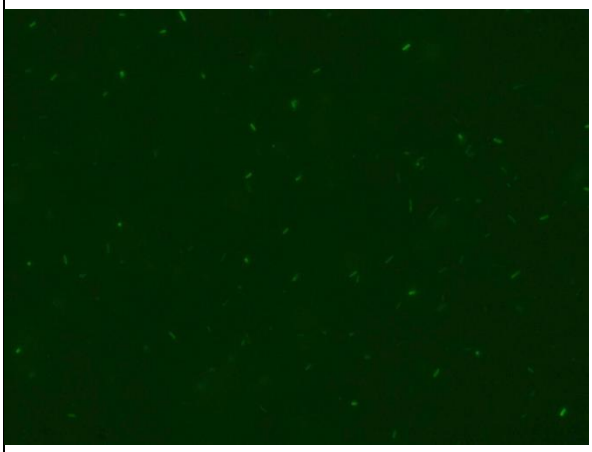


(d)

Figure S6. *B. subtilis* loaded with Newport Green™ DCF and treated with $CoCl_2$ (a and b) or HEPES (c and d). Images are brightfield (a) and fluorescence (b) of Newport Green™ DCF loaded *B. subtilis*, treated with 100 µM $CoCl_2$; and brightfield (c) and fluorescence (d) of Newport Green™ DCF loaded *B. subtilis*, treated with HEPES as negative control.



(a)



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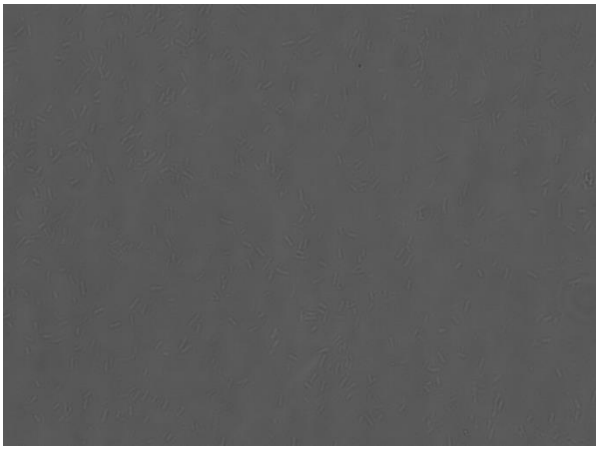

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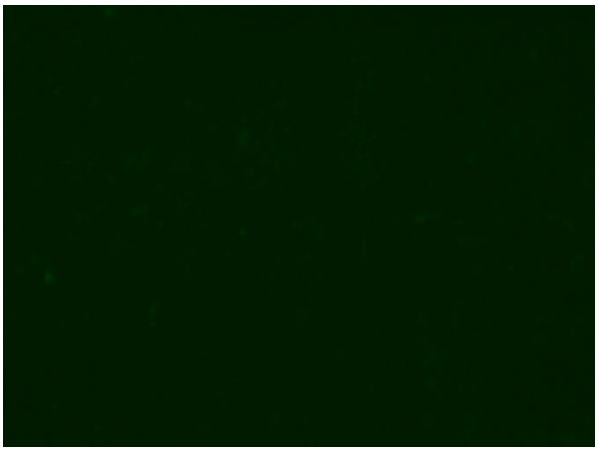

(d)

Figure S7. *S. oneidensis* MR-1 loaded with Newport Green™ DCF and treated with $CoCl_2$ (a) and (b) or HEPES (c and d). Images are brightfield (a) and fluorescence (b) of Newport Green™ DCF loaded *S. oneidensis*, treated with 100 µM $CoCl_2$; and brightfield (c) and fluorescence (d) of Newport Green™ DCF loaded *S. oneidensis*, treated with HEPES as negative control.

Resin-embedding for TEM of biological samples

To prepare bacteria for biological TEM, colonies of *S. oneidensis* MR-1 or *B. subtilis* were grown on LB agar plates overnight at 30 or 37 °C, respectively, and then transferred into 10 mL of LB broth. The bacteria were then incubated in an orbital shaker (300 RPM) overnight until bacteria reached the late log phase (OD_{600} > 1). Bacteria were washed by centrifugation at 750 × g for 10 minutes, removal of supernatant, and resuspension in D-PBS. This was repeated to suspend the bacteria in HEPES buffer. The optical density of this solution was adjusted to be between 0.6 and 1. Meanwhile, working solutions of 1000, 100, 10, and 1 mg/L along with particle-free HEPES as a negative control were prepared as described above. In 1.7 mL microcentrifuge tubes, 900 µL of the bacterial stock was mixed with 100 µL of the working solutions. The exposed bacteria were incubated for 1 hour, followed by centrifugation for 5 minutes at 800 x g. The supernatant was removed, and the pellet was washed with 0.1 M cacodylate buffer by adding it to the surface of the pellet—allowing it to sit for 5 minutes, followed by 2 minutes of centrifugation at 500 × g. This was repeated twice. The supernatant was then removed and the pellet was resuspended in 2.5% glutaraldehyde in 0.1 M cacodylate buffer, and allowed to fix for 50 minutes. After 25 minutes had elapsed, the pellet was manually flipped using sterile wooden inoculation sticks, which had been snapped to yield thin, sharp ends. After the 50 minutes of fixation, the tubes were centrifuged at 800 × g for 5 minutes. The glutaraldehyde-containing buffer was removed, and the pellet was washed three times with 0.1 M cacodylate buffer, with 5-minute waiting periods between washes.

Following fixation, the pellets were dehydrated using sequential washes with increasing ethanol content up to 100% ethanol. After dehydration, the pellet was washed with propylene oxide three times, with 3 minute rests between washes. To this washed pellet was added a 2:1 propylene

oxide:epoxy resin (9.66 g Polybed812, 5.055 g DDSA, 4.02 g NMA, and 0.375 mL DMP). After 2 hours of soaking, this resin was replaced with 1:1 propylene oxide/epoxy resin and allowed to sit overnight. This resin was replaced with fresh 1:1 propylene oxide/epoxy resin and allowed to sit, covered, for 4 hours. Finally, pure epoxy resin replaced the 1:1 resin and sat overnight. Resin was removed, replaced with fresh resin, and the samples were transferred to a 40 °C oven for curing for 24 hours, then to 60 °C oven to finish curing. Samples were microtomed and stained before being placed on TEM grids for imaging.

Supporting Information References

1. Ge, L.; Han, C.; Xiao, X.; Guo, L., In situ synthesis of cobalt–phosphate (Co–Pi) modified g-C_3N_4 photocatalysts with enhanced photocatalytic activities. *Applied Catalysis B: Environmental* **2013,** *142*, 414-422.
2. Chuang, T.; Brundle, C.; Rice, D., Interpretation of the x-ray photoemission spectra of cobalt oxides and cobalt oxide surfaces. *Surface Science* **1976,** *59* (2), 413-429.