Supplementary Information

Nanoscale battery cathode materials induce DNA damage in bacteria

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Table S1. A list of relevant previous toxicology studies.

Reference	Chemicals or nanomaterials	Biological organisms	Toxicity insights
Soil Biology and Biochemistry 1998, 30, 10-11, 1389-1414	Heavy metals	Soil microorganisms	A general review on toxicity of heavy metals on soil microorganisms
Environmental Pollution 2011, 159, 7, 1749-1756	Commercial CuO and ZnO NPs	A soil bacterium, *Pseudomonas chlororaphis* O6	Both metal ions and ROS contribute to nano metal oxide toxicity.
ACS Nano 2012, 6, 6, 5164–5173	TiO_2, ZnO, CeO_2, Fe_2O_3, SiO_2, Al_2O_3 and CuO NPs	*Escherichia coli*	ROS level caused by NPs has a linear correlation to NP antimicrobial activity, except for CuO, which releases bactericidal Cu^{2+}.
Environ. Sci. Technol. 2015, 49, 2, 1105–1112	24 metal oxide NPs	*Escherichia coli*	Probability of a metal oxide NP being toxic increases as the hydration enthalpy becomes less negative and as the conduction band energy approaches those of biological molecules.
J. Environ. Sci. 2013, 25, 5, 882-888	ZnO, CuO, Co_3O_4 and TiO_2 NPs	*Escherichia coli*	Both ROS and metal ion release contribute to the toxicity from metal oxide NPs. ROS is correlated to cellular response in oxidative stress.
Current Pharmaceutical Design 2018, 24, 8, 896-903	Metal oxides	Various bacteria	A review on challenges and interpretations of antibacterial metal oxide nanoparticles.
J. Nanobiotechnology 2016, 14, Article number: 73	Pure and multi metal oxide NPs	Various bacteria and mammalian cells	A review on the antibacterial and cytotoxic properties of metal oxides. Mechanisms for antibacterial properties include binding to cell wall, ROS, and metal ion release.
Environ. Sci. Technol. 2017, 51, 17, 10137–10145	Nano- and bulk particles (TiO_2, ZnO, V_2O_5, CeO_2, Fe_2O_3, and Al_2O_3)	*Photobacterium phosphoreum* 502	The toxicity effect was attributed to the long-lived $O_2^{\bullet-}$ radicals on the nanoparticle, and its potency follows the order of $TiO_2 > ZnO > V_2O_5 > Fe_2O_3 > CeO_2 > Al_2O_3$, which is the same as the order of the $O_2^{\bullet-}$ concentration
J. Nanoparticle Research 2010, 12, 1531-1551	Silver nanomaterials	Bacteria	Silver NP toxicity mechanisms include (1) silver ion release and ROS, (2) interaction with membrane, and (3) cell entrance and interaction with DNA.
Chemosphere 2009, 79, 1, 113-116	Al_2O_3, Co_3O_4, CuO, TiO_2, and ZnO NPs	*Escherichia coli, Salmonella typhimurium*	CuO displays low mutagenic potential to *Salmonella typhimurium* strains at specific concentrations.
Environmental Pollution 2012, 169, 81-89	CuO NPs	*Escherichia coli*	Nano-CuO induces ROS formation and single-stranded DNA damage. Cu ions released by CuO NPs mainly contribute to the ROS and DNA damage response.
Free Radical Biology and Medicine 2011, 51, 10, 1872-1881	ZnO and TiO_2 NPs	*Escherichia coli*	NPs induce ROS and oxidative stress response in cells, plus DNA fragmentation.
…			
Our study	Nanoscale complex metal oxide ($Li_xNi_yMn_zCo_{1-y-z}O_2$, NMC)	*Shewanella oneidensis*, *Bacillus subtilis*	DNA strand breakage and increased chemical modification to DNA molecules are observed after nano-NMC exposure, and such DNA damage is related to ROS and metal ion release.

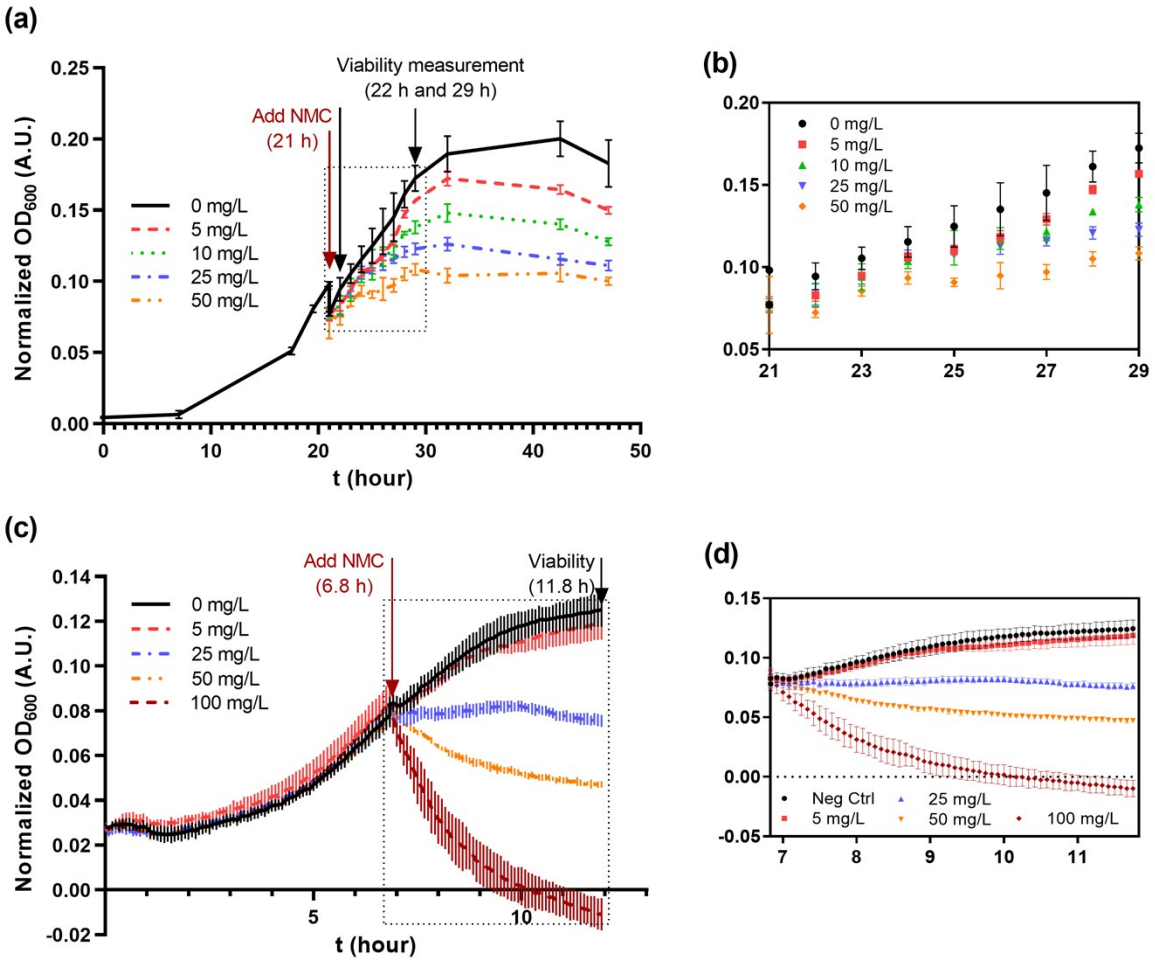


Figure S1. Dosing NMC at mid-log phase of (a) *S. oneidensis* MR-1 and (c) *B. subtilis* growth with error bars and magnified view of exposure window (b) and (d). Error bars denote standard deviation (n=3).

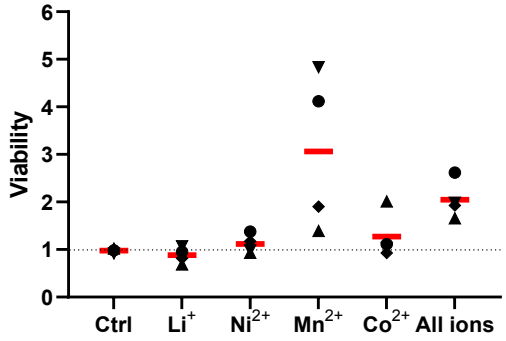


Figure S2. Viability measured via growth-based viability (GBV) test after *B. subtilis* were exposed for 5 hours to ions at concentrations equivalent to those released from 5 mg/L NMC.[1] Solid shapes represent averages of four different experimental runs and red line represents average of experimental replicates.

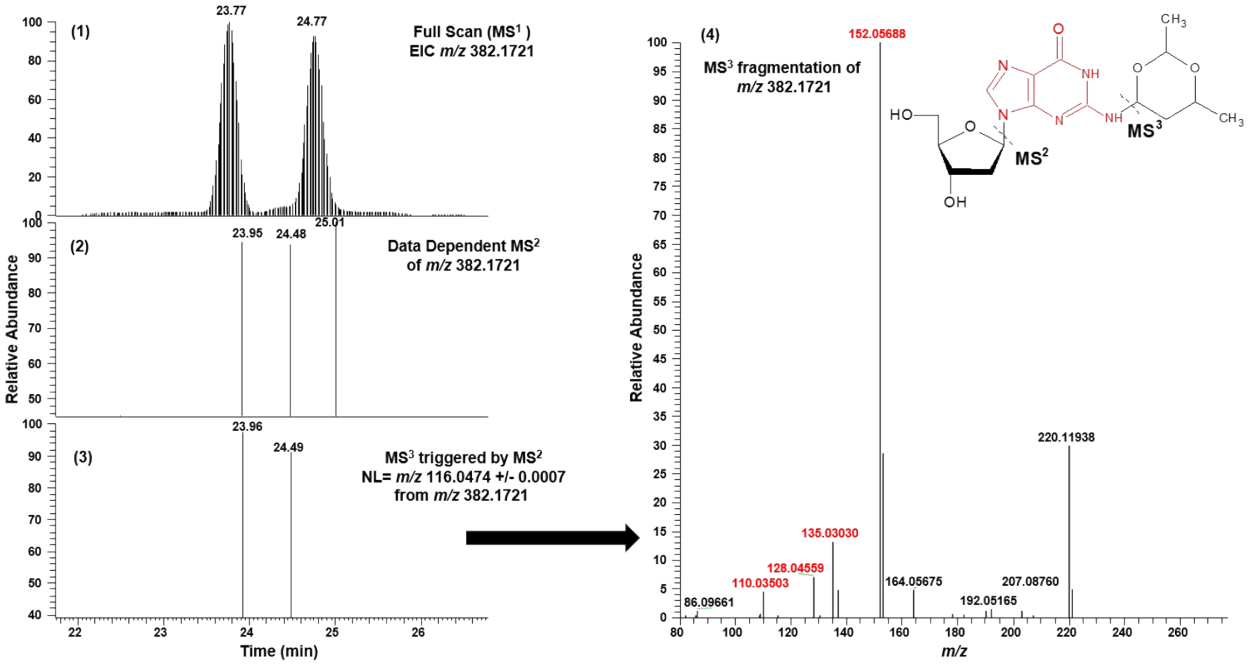


Figure S3. High resolution accurate mass (HRAM) mass spectrometry DNA adductomic approach triggering MS^3 fragmentation by neutral loss (NL) monitoring of mass corresponding to deoxyribose. Here we show the detection of a putative DNA adduct, N^2-dimethyldioxane-dG. Panel 1: Extracted ion chromatogram (EIC) from full scan of *m/z* 382.1721, Panel 2: MS^2 scan event of *m/z* 382.1721, and Panel 3: MS^3 scan event triggered by a mass difference of 116.0474 amu between the *m/z* 382.1721 in the Full Scan spectrum and the corresponding triggered MS^2 spectrum demonstrating the presence of an adduct; Panel 4: Putative structure, MS^2 and MS^3 fragmentation and MS^3 spectrum of *m/z* 382.1721 (*m/z* in red: diagnostic fragments of guanine).

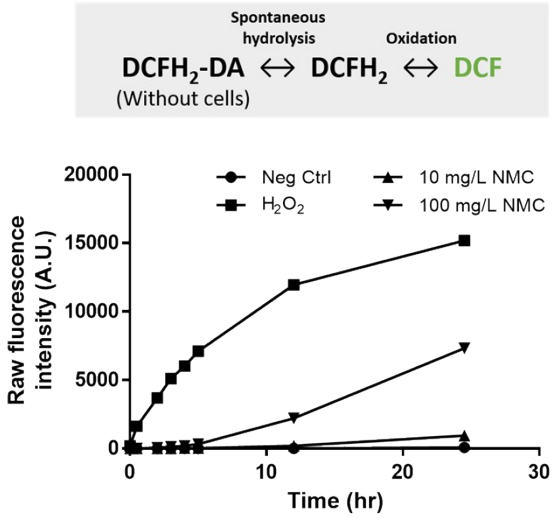


Figure S4. Abiotic generation of ROS from nano-NMC exposure in bacterial growth minimal medium. $DCFH_2$-DA is a cell-permeant dye with two acetate groups, which are hydrolyzed by esterase in cells upon entering cells. The resultant non-fluorescent 2',7'-dichlorodihydrofluorescein ($DCFH_2$) is then oxidized by intracellular ROS and converted to highly fluorescent 2',7'-dichlorofluorescein (DCF).[2] This conversion can also happen abiotically and spontaneously.[3]

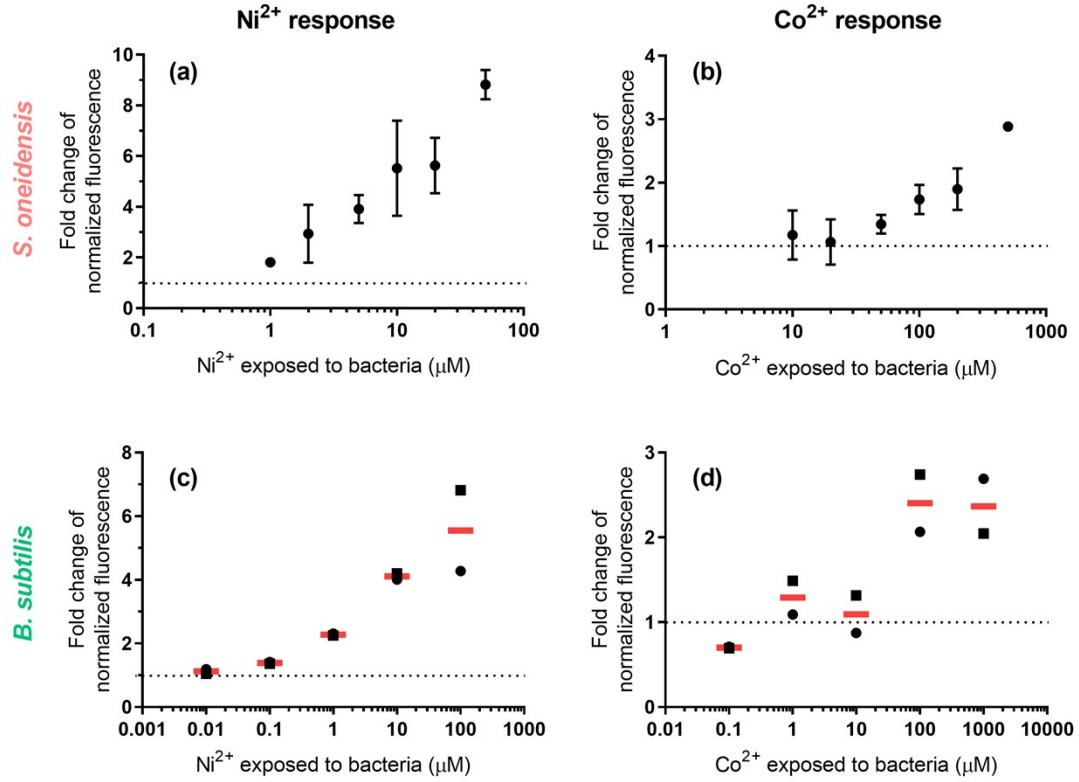
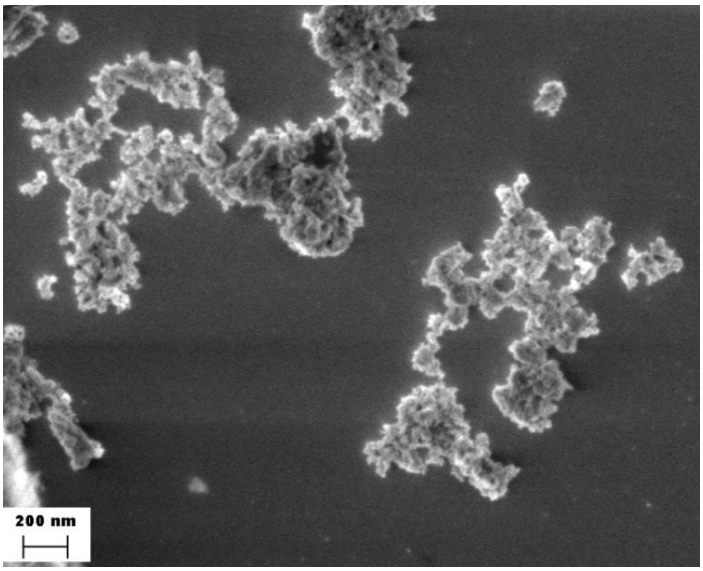


Figure S5. Intracellular fluorescence increased upon Ni^{2+} and Co^{2+} exposure to bacteria. For *S. oneidensis*, results from one experimental run containing biological triplicates are presented; error bars represent standard deviation. For *B. subtilis*, results from two experimental run each containing two biological replicates are shown; each data point stands for the average of one run, and the red bar represents an average of experimental runs.

Methods

Materials

Nanoscale NMC materials: Nano-NMC were synthesized as described in our previous publication.[4] Briefly, equimolar amounts of the Ni, Mn, and Co salts were introduced into an aqueous solution of 0.1 M LiOH, producing a precipitate of $Ni_{1/3}Mn_{1/3}$ $Co_{1/3}(OH)_2$. After repeated washing, the precipitate was dried in a vacuum oven at 40°C. The samples were then added to a molten salt flux (6:4 molar ratio of $LiNO_3$:LiOH) at 205°C in a poly(tetrafluoroethylene) container for 30 minutes. The molten salt was quenched with water, clean and rinsed by multiple centrifugation steps, and dried for 2 days at 40°C. The figure at right shows a representative scanning electron microscope image of the resulting nanoparticles deposited onto a Si substrate, from the batch of nanoparticles used in the studies reported here. This image, and additional images (not shown) are consistent with additional SEM, transmission electron microscopy, and atomic force microscopy measurements we reported previously on samples we prepared using this identical synthesis method.[4] The ζ-potential of NMC when dispersed into bacterial liquid medium was determined to be -9.3 ± 0.8 mV in *S. oneidensis* growth medium (Brookhaven ZetaPALS) and -15.2 ± 0.6 mV in *B. subtilis* growth medium (Malvern Zetasizer Nanoseries).



Chemicals and reagents: Difco™ LB broth (Miller) and Difco™ agar (granulated) were purchased from Becton, Dickinson, and Company. 2′,7′-Dichlorofluorescin diacetate ($DCFH_2$-DA) was purchased from both Sigma Aldrich (Cat. No.: D6883) and ThermoFisher Scientific (Cat. No.: D399). Newport Green DCF diacetate was purchased from ThermoFisher Scientific (Cat. No.: N7991). $NiSO_4 \cdot 6H_2O$ was purchased from Acros Organics (Cat. No.: B00875). $CoCl_2 \cdot 6H_2O$ was purchased from Mallinckrodt (Cat. No.: B00791). Anhydrous DMSO was purchased from ThermoFisher Scientific (Cat. No.: D12345). Hydrogen peroxide (30% w/w) was purchased from Fisher Scientific (Cat. No.: H325-100). Dulbecco's phosphate-buffered saline (DPBS) was purchased from Mediatech, Inc. All Milli-Q water used had a resistance around 18.0 MΩ·cm. Tris-EDTA (TE) buffer was composed of 10 mM Tris, 1 mM EDTA, brought to pH 8.0 with HCl. Lysozyme was purchased from Sigma Aldrich (Cat. No.: L6876). Isopropanol (IPA) was purchased from ThermoFisher Scientific. Tris-$MgCl_2$ buffer was composed of 10 mM Tris, 5 mM $MgCl_2$, brought to pH of 7.

Medium preparation

LB broth: 25 g of pre-mixed Difco LB power was dissolved in 1 L of Milli-Q water and autoclaved.

Minimal medium with sodium lactate for S. oneidensis (MM with lactate): Each liter of minimal medium contains 0.68 g NaCl, 0.3 g KCl, 0.285 g $MgCl_2 \cdot 6H_2O$, 0.3975 g Na_2SO_4, 0.15 g NH_4Cl, 2.383 g HEPES, 0.0125 g Na_2HPO_4 and 0.0056 g $CaCl_2$. Part I stock contains 20x concentration of NaCl, KCl, $MgCl_2 \cdot 6H_2O$, Na_2SO_4, NH_4Cl, and HEPES. Part II stock contains 100x concentration of Na_2HPO_4 and Part III stock contains $CaCl_2$, both sterile filtered. To make 1 liter of 1x minimal medium solution, 50 mL Part I stock was mixed with 930 mL Milli-Q water, and pH was adjusted to 7.2~7.3 using HCl and NaOH solutions. The solution was autoclaved and cooled down to room temperature, and 10 mL of both Part II and Part III stock was added;

this solution is called minimal medium (MM) in this study. Immediately before use, 14 mL of sodium DL-lactate syrup 60% w/w aq. solution was mixed with the minimal medium to make a total volume of 1000 mL, referred to as MM with lactate.
Minimal medium with dextrose for B. subtilis (MM with dextrose): The same minimal medium (MM) as for *S. oneidensis* was used. Immediately before use, 1 M dextrose syrup was mixed with the minimal medium to make a solution of 10 mM dextrose, referred to as MM with dextrose.

Abiotic ROS generation from NMC
$DCFH_2$-DA from Sigma Aldrich was dissolved in anhydrous DMSO to a concentration of 20 mM, divided into 100 µL aliquots and stored at -20 °C in a desiccator. Upon usage, $DCFH_2$-DA DMSO stock was thawed and diluted 1000-fold into MM with lactate. NMC was weighed and dissolved in freshly prepared MM with sodium lactate with a stock concentration of 4 mg/mL. The prepared NMC suspension was aged for 10 days on a bench, and part of the NMC stock suspension was diluted 10-fold using fresh MM with lactate. On a black wall clear bottom 96-well assay plate, 195 µL of diluted $DCFH_2$-DA solution was added into all 96 wells. At time zero (t = 0), 5 µL of NMC suspensions of two concentrations plus negative control (MM with lactate) and positive control (30% w/w hydrogen peroxide solution) were quickly added in triplicate to the first row of wells that already contained diluted $DCFH_2$-DA, and the plate was immediately sent for fluorescence reading at excitation/emission (Ex/Em) of 485/525 nm on a Synergy 2 Multi-Mode Microplate Reader (BioTek, VT). Every 30 minutes, the same blank, NMC suspension, and hydrogen peroxide solution were added to a new row and immediately sent for fluorescence reading.

DNA extraction from bacterial cells
DNA extraction of *S. oneidensis* was done using Gentra Puregene Yeast/Bacteria Kit (Qiagen), and the protocol was optimized to extract bacterial DNA with high yield for the adductomics study. Bacterial cells were harvested via centrifugation. The resulting pellets were suspended in 20 mg/mL lysozyme solution in TE buffer (approximately 300 µL per 1 x 10^9 cells) and incubated at room temperature (RT) for 10 minutes while shaking. Lysozyme-treated cells were pelleted via centrifugation for 1 min at 16,000 × g at 4 °C, followed by careful removal of supernatant with a micropipet. 300 µL of Cell Lysis Solution was added per pellet and mixed with cells by pipetting up and down. The mixture quickly became sticky, and the pellet was partially resuspended. After 5 minutes of rest at RT, 1.5 µL RNase A Solution from the kit was added and mixed by inverting the tubes 25 times. The mixtures were incubated at RT for 2 hours without shaking. Samples were then quickly cooled down on ice for 1 minute, followed by addition of 100 µL Protein Precipitation Solution and vigorous vortexing for 20 seconds at high speed. The samples were then incubated on ice for 10 minutes and centrifuged for 3 minutes at 16,000 × g at 4 °C. In new, clean 1.5 mL microcentrifuge tubes, the supernatant was carefully poured into 300 µL isopropanol (IPA), and the resulting solution was gently mixed by slowly inverting tubes 50 times. White floating substances, the DNA, started to appear during mixing. To collect precipitated DNA, the mixture was centrifuged for 1 min at 16,000 × g at 4 °C, followed by two washing steps using 300 µL of 70% and 100% IPA. After careful removal of supernatant, tubes containing DNA at bottom were drained on a clean piece of absorbent paper and allowed to air dry. To measure concentration, DNA pellets were dissolved in Tris-$MgCl_2$ buffer overnight in a 4 °C fridge, and the concentration was measured using a NanoDrop One spectrometer with a standard of A260/A280 ~1.8.
Genomic DNA of *B. subtilis* was extracted using a DNeasy PowerLyzer Microbial Kit (Qiagen), following the manufacturer protocol with modifications for gram positive bacterial DNA for adductomics study. Briefly, NMC-exposed bacterial cells were harvested, and suspended in 1.8 mL of TE buffer (per ~10^8 cells). Following the manufactural protocol coupled with the beading cycle of 10 M/s of 5 cycle for 300 seconds using a FastPrep-24® 5G Homogenizer, bacterial cells were effectively lysed. Upon protein precipitation with the IRS solution in the kit, cell lysate was loaded onto the MB spin column. After washing the column

with the CB buffer from the kit, DNA samples were finally eluted from the spin column in a Tris-$MgCl_2$ buffer (Tris HCl/$MgCl_2$ (10mM/5mM), pH 7: 15.5 mg Trizima + 10.2 mg $MgCl_2$ $6H_eO$ + 10 mL MilliQ H_2O).

NMC exposure to bacterial cells for DNA adductomics

S. oneidensis protocol: The exposure was done similar to that in the bacterial growth assay. Instead of using varying concentrations of NMC, only the selected 5 mg/L exposure concentration was used in addition to the negative control. Three replicates were used in the experiment. After being grown in large volume in a flask to mid-log phase, 21.9 mL bacterial suspension was aliquoted into 50 mL sterile conical tubes, followed by addition of freshly prepared NMC suspension. Bacterial growth was monitored over time by transferring 200 µL aliquots and measuring OD_{600}. At 8-hour post-exposure, an additional 200 µL aliquot was used for a growth-based viability assay, and the rest of bacterial suspensions were centrifuged at 4,000 × g for 20 minutes. Cell pellets were saved at -80 °C until being thawed for DNA extraction, followed by sample preparation for DNA adductomics.

B. subtilis protocol: The procedure was similar to that of *S. oneidensis*, only in a larger scale. After 300 mL bacterial culture was being grown in a 1 L flask until mid-log phase, 135 mL of cell cultures were distributed into two 500 mL flasks, and 15 mL of 10X NMC working solution or fresh MM with dextrose was added to bacterial cells. Bacterial growth was monitored by transferring 500 µL aliquots and measuring OD_{600} in a visible spectrometer (SpectroVis® Plus, Vernier Software and Technology). After the 5-hour exposure to 5 mg/L NMC, the bacterial suspension was centrifuged at 4,000 × g for 20 minutes. Cell pellets were saved at -80 °C until being thawed for DNA extraction, followed by sample preparation of DNA adductomics.

RNA Extraction

A Direct-zol™ RNA MiniPrep kit (Zymo Research) was used to extract RNA from both *S. oneidensis* and *B. subtilis* cells. For *S. oneidensis*, bacterial cells were harvested after either 8 hours for 5 mg/L or 1 hour for 50 mg/L NMC exposure. For *B. subtilis*, the NMC exposure was either 5 hours for 5 mg/L or 1 hour for 50 mg/L NMC. After centrifugation, bacterial cells were re-suspended in RNAzol® RT solution (Molecular Research Center, Inc.). For *S. oneidensis*, cells were simply re-suspended and lysed by pipetting up and down. For *B. subtilis*, a bead beater was used to facilitate cell lysis. Cells were homogenized using FastPrep-24™ 5G Homogenizer (MP Biomedicals) at 10 M/S, 5 cycle of 300 seconds, pause of 100 seconds between cyclewith 0.1 mM RNAse free glass beads (Next Advance Inc., GB01-RNA) in RNAzol® RT solution. Total RNA was extracted from both lysed bacterial cells using manufacturer's protocol from Zymo Research with an on-column DNase I treatment at 30 °C for 15 minutes. RNA was finally eluted with nuclease-free water from the spin column at 16,000 g for 1 minute. RNA characterization was done using a NanoDrop™ One/One^C Microvolume UV-Vis Spectrophotometer using Nucleic Acid 260/280 Ratio. The total isolated RNA sample were then preserved in at -80˚C until total reverse transcription.

Sample preparation for DNA adductomics

DNA digestion. Concentration of DNA dissolved in Tris-$MgCl_2$ buffer was quantified using a UV/vis spectrophotometer. Afterwards, the DNA was incubated with 30 mg of $NaBH_3CN$, in order to stabilize possibly formed Schiff Bases. $NaBH_3CN$ was removed through filtration by using a double filtration membrane Amicon Ultra (30 kDa cutoff, 0.5 mL) and washed with 1.2 mL of Tris buffer. The desalted DNA was reconstituted with 300 µL of Tris and transferred from the filtration membrane into a clean reaction vial. The DNA recovery from the filtration membrane was assessed using a UV/Vis spectrophotometer. A two-step DNA digestion was performed to digest macromolecules into single nucleosides using DNase (from *E. coli*, Aldrich), phosphodiesterase-1 (PDE-1) (from *Crotalus adamanteus*, Aldrich) and alkaline phosphatase (ALP) (from *Pichia pastoris*, Aldrich). All enzymes were purified using a double filtration membrane from Amicon Ultra (0.5 mL, cutoff 10 k Da) prior to use. The first step of hydrolysis was done using only DNase, followed by a second step using the full enzyme mixture. Both steps incubated samples for 24 hours at RT. Per 1 µg of DNA, 0.5 Units of DNase was used in the first step, and the second step

used 0.5 U, 0.2 U and 0.02 mU of DNase, ALP and PDE-1, respectively. After the two-step hydrolysis, enzymes were removed from the mixture by using an Amicon Microcone single filtration membrane (0.5 mL, cutoff 10 kDa), previously washed for three times with water to decrease the presence of PEG. The digestion yield was assessed by measuring the concentration of dG via an LC/UV measurement described in the next paragraph.

dG quantitation. A Dionex UltiMate 3000 RSLCnano System (Thermo Scientific, Waltham, MA) equipped with a reverse phase column (Luna C18, 250x0.5 mm, 5 µm, 100 Å) was used to separate the four 2'-deoxyribonucleosides (dC, dG, dT, dA) and quantify the amount of dG. A gradient of two mobile phases, H_2O (A) and methanol (MeOH, B), was used. Operated at 40 °C with a flow rate of 15 µL·min-1, the system started elution with an isocratic step at 5% B for 3 minutes, followed by a first linear gradient of 0.58 % B·min^{-1} (12 min), a second linear gradient of 27.67% B·min^{-1} (3 min), and it concluded with a second isocratic step at 95% B (3 min). The column was re-equilibrated for 9 minutes using 5% B. Absorbance at 254 nm was used to monitor elution. Using a calibration curve consisting of eight standard concentrations (0.0625, 0.125, 0.25, 0.50, 1.00, 2.00, 4.0, 8.0 ng/µL pf dG), the amount of dG in digested DNA samples was quantified. For each sample 3µL were injected.

Hydrophobic reversed phase fraction collection. To exclude signals from unmodified deoxyribonucleosides (dC, dG, dT, dA) and enrich analytes of interest (i. e. DNA adducts) for the following LC/MS^3 measurement, separation and fraction collection using chromatography was performed, followed by sample concentration and reconstitution. An HPLC (Ultimate 3000, Thermo Scientific, Waltham, MA) equipped with a C18-Column (4.6 x 250 mm, 100 Å, 5 µm Luna-Phenomenex, Torrace, CA) was used for fraction collection, using mobile phase H_2O (A) and MeOH (B). For hydrolyzed DNA samples extracted from NMC-exposed cells, fraction collection was run at 4°C with a flow rate of 1.0 mL·min^{-1}.The elution program involved an isocratic step at 2% of B (5 min), followed by a series of linear gradients of 0.86% B·min^{-1} (6 min), 0.14% B·min^{-1} (28 min), 4.6% B·min^{-1} (5 min), 13% B·min^{-1} (5 min), and an isocratic step at 100% of B (5 min). At the end of the elution, the LC-system was equilibrated in isocratic conditions (2% of B) for 20 min. Two different wavelengths (190 nm and 254 nm) were used to monitor elution. Fraction collection started at 10 min and ended at 60 min of the program, and only fractions between nucleoside peaks were collected. Collected fractions from one sample were combined, dried in a SpeedVac concentrator, and stored at -20 °C until LC/MS^3 adductomic analysis. Two isotopic standards, ^{15}N-*N*^{6}-MethyldA and ^{15}N-*N*^{2}-EthyldG, were added during DNA digestion and sample concentration for the purpose of quality control. For DNA from direct NMC exposure, two additional isotopically labeled standards, D_4-POB-dT and D_4-POB-dG were also used.[28]

LC/MS^3 adductomic analysis

The dried DNA samples were reconstituted in 20 µL of LC-MS water (LCMS grade, Fluka) and then analyzed with a nanoflow UPLC system (Ultimate 3000 RSLCnano, Thermo Scientific, Waltham, MA) coupled to a hybrid Orbitrap mass spectrometer (Fusion Tribrid, Thermo Scientific, Waltham, MA). 2 µL were injected for each sample. The UPLC system operated with a 5-µL loop. The chromatographic separation was performed with a RP-column created by hand packing a commercially available fused-silica emitter (230 x 0.075mm, 10 µm orifice, New Objective, Woburn MA) with C18 stationary phase (5 µm, 100Å, Luna C18, Phenomenex, Torrance, CA). The mobile phase consists of water with 0.05 %v/v formic acid (phase-A) and acetonitrile (phase-B). The elution program involved an isocratic step (2 % of B for 5 min at 1µL·min^{-1}), followed by a linear gradient of B (1.5 %·min^{-1} for 25 min at 0.3 µL·min^{-1}), and it concluded with a washing isocratic step, performed at 98% of B for 5 min at 0.3µL·min^{-1}. At the end of the elution program, the LC-system was equilibrated for 5 min in isocratic conditions (2% of B, 1 µL·min^{-1}). During the LC run, the injection valve was switched at 6 min, removing the sample loop from the liquid flow path to minimize gradient delay and allow for washing of the sample loop. The LC system was interfaced to the MS-detector

using a nanoflow ESI ion source (Nanoflex, Thermo Scientific, Waltham, MA) operated in positive ion mode at 2.5 kV and with an ion transfer tube temperature of 350 °C.

The MS analyses consist of three detection events: full scan, targeted data dependent MS^2-acquisition (dd-MS^2), and a neutral loss MS^3 data acquisition (NL-MS^3) all performed with Orbitrap detection. An inclusion list of 128 DNA adducts was used to trigger MS^2 scan events. The full scan (100-600 m/z) detection was performed with quadrupole isolation, maximum injection time of 50 ms, automatic gain control (AGC) setting of 200000, and a resolution of 120000 (ref. 400 m/z). MS^2 fragmentation was performed in the ion trap on full scan ions which were within 5 ppm of the inclusion list masses (up to 5, based on abundance) and with intensities greater than 10^3 counts, using quadrupole isolation of ± 1.5 *m/z,* data dependent dynamic exclusion of 20 s, 30% CID collision energy, 10 ms activation time, 200ms maximum injection time, AGC setting of 200000, and Orbitrap detection at a resolution of 60000 (ref. 400 m/z). MS^3 data acquisition was performed on MS^2 product ions which were different from their parent ions by the mass of the deoxyribose moiety (-dR; 116.0474 ± 0.0006 m/z, 5ppm) using an isolation width of ± 3.0 m/z, maximum injection time of 300 ms, AGC setting of 50000, 50% HCD fragmentation and at a resolution of 15000 (ref. 400 m/z).

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