

Supporting Information

Towards resolution of antibacterial mechanisms in metal and metal oxide nanomaterials: a meta-analysis of the influence of study design on mechanistic conclusions

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Table S1. Search terms used to query Web of Science, by core composition. All searches were performed during July 2019. Filters were set to include documents of the type “Article” published as early as 2000 in the English language. “TS” indicates that terms were searched for in the title, abstract, and keywords of papers.

ENM core composition	Search term
Cu/CuO	TS=((nano* AND (Cu OR *CuO OR *copper)) AND ((mechanism\$ OR mode of OR pathway) AND (*microb* OR *toxic* OR *bacteri*)))
TiO ₂	TS=((nano* AND (*TiO2 OR *titanium dioxide)) AND ((mechanism\$ OR mode of OR pathway) AND (*microb* OR *toxic* OR *bacteri*)))
Ag	TS=((nano* AND (Ag OR *silver)) AND ((mechanism\$ OR mode of OR pathway) AND (*microb* OR *toxic* OR *bacteri*)))
ZnO	TS=((nano* AND (*ZnO OR *zinc)) AND ((mechanism\$ OR mode of OR pathway) AND (*microb* OR *toxic* OR *bacteri*)))

Table S2. Full list of study design and conclusion variables within each category (see Table 2 of the main text). The “Variable as Statement” column gives the statement that must apply to a study for the value of the variable in question to be recorded as “positive” for that study. Studies to which this statement does not apply are recorded as “negative” for that variable.

	Variable Category	Category Notes	Variable Name	Variable as Statement
Study Design Variables	ENM size	dry size, as measured by TEM/SEM	<10nm	The ENMs used were smaller than 10nm in their smallest dimension, as measured by TEM or SEM
			10-20nm	The ENMs used were between 10 and 20nm in their smallest dimension, as measured by TEM or SEM
			20-30nm	The ENMs used were between 20 and 30nm in their smallest dimension, as measured by TEM or SEM
			30-40nm	The ENMs used were between 30 and 40nm in their smallest dimension, as measured by TEM or SEM
			40-50nm	The ENMs used were between 40 and 50nm in their smallest dimension, as measured by TEM or SEM
			50-100nm	The ENMs used were between 50 and 100nm in their smallest dimension, as measured by TEM or SEM
			Compare to >100nm	ENMs were compared to materials of particle size larger than 100nm in their smallest dimension, as measured by TEM or SEM
			Compare multiple	ENMs of multiple sizes were compared in mechanism-targeted experiments
			Unspecified	The size of ENMs was not specified
			Surface coating	applies only to colloidal ENMs
	Uncapped	The ENMs used were (reported to be) uncoated with any nonmetallic capping or reducing agent		
	Compare	Coated and uncoated ENMs were compared		
	Unspecified	No comment was given on the presence or absence of capping or reducing agents		
	Tox control	The antibacterial activity of the capping or reducing agent(s) alone (without ENMs) was assessed during toxicity assessment		
	Multiple capping agents	Multiple capping or reducing agents were compared in mechanism-targeted experiments		

Surface charge	measured by zeta potential, in exposure medium	Positive	ENMs had a positive surface charge in the exposure media used for mechanistic studies
		Negative	ENMs had a negative surface charge in the exposure media used for mechanistic studies
		Compare multiple	ENMs with different surface charges were compared in mechanistic studies
		Unspecified	The surface charge of ENMs was not specified
Mode of delivery	colloidal or immobilized ENMs	Colloidal	ENMs were colloidal (suspended in liquid)
		Immobilized	ENMs were immobilized (embedded in a matrix or on a membrane)
		Compare multiple	Colloidal and immobilized ENMs were compared in mechanistic studies
		Unspecified	The mode of delivery of ENMs was not specified
Bacterium Gram type		Gram positive	The bacterium used for mechanistic studies was a Gram positive bacterium
		Gram negative	The bacterium used for mechanistic studies was a Gram negative bacterium
		Both	Both Gram positive and Gram negative bacteria were used for mechanistic studies
		Not specified	The bacterium used for mechanistic studies was not specified (or the study was done on a diverse community of many bacteria)
Characterization		SEM/TEM	SEM or TEM was used to characterize the ENMs for this study
		DLS	DLS was used to characterize the ENMs for this study
		Zeta potential	The zeta potential of ENMs was characterized in this study
		XRD	XRD was used to characterize the ENMs for this study
		BET	BET was used to characterize the ENMs for this study
		AFM	AFM was used to characterize the ENMs for this study
		XPS	XPS was used to characterize the ENMs for this study
		UV-Vis	UV-Visible spectroscopy was used to characterize the ENMs for this study
FTIR	FTIR was used to characterize the ENMs for this study		

Toxicity assay	the experimental method(s) used to assess the magnitude of antibacterial activity (not the mechanism)	ICP or AAS	ICP-MS (or -OES, -AES) or AAS was used to characterize the ENMs for this study
		EDS	EDS was used to characterize the ENMs for this study
		PL Spectroscopy	PL spectroscopy was used to characterize the ENMs for this study
		CFU count/plating	A CFU count/plating assay was used to characterize the magnitude of antibacterial activity
		KB/Disk diffusion	The Kirby Bauer/disk diffusion assay was used to characterize the magnitude of antibacterial activity
		Broth microdilution/growth inhibition in liquid media	The broth microdilution assay was used to characterize the magnitude of antibacterial activity
		Membrane	A membrane damage assay was used to characterize the magnitude of antibacterial activity
		Other	Another assay was used to characterize the magnitude of antibacterial activity
		Multiple concentrations	The antibacterial activity of ENMs was assessed at multiple concentrations
		Time points	The antibacterial activity of ENMs was assessed at multiple time points
Lighting	for mechanism-targeted experiment(s)	UV	Mechanism-targeted experiments were conducted under UV illumination
		Visible	Mechanism-targeted experiments were conducted under visible light
		Dark	Mechanism-targeted experiments were conducted in darkness
		Compare multiple	Multiple lighting conditions were compared in mechanism-targeted experiments
		Unspecified	Lighting conditions were not specified
ENM aggregation	hydrodynamic size, measured by DLS, applies only to colloidal ENMs	Control	A negative control (without ENMs) was used in the mechanism-targeted experiment with the same illumination
		<50nm	The hydrodynamic size of ENMs was less than 50nm

Conclusion Variables

Mechanism-targeted methods

50-100nm	The hydrodynamic size of ENMs was between 50 and 100nm
100-200nm	The hydrodynamic size of ENMs was between 100 and 200nm
200-500nm	The hydrodynamic size of ENMs was between 200 and 500nm
>500nm	The hydrodynamic size of ENMs was greater than 500nm
Compare multiple	ENMs of multiple hydrodynamic sizes were compared in mechanism-targeted experiments
Unspecified	The hydrodynamic size of ENMs was not specified
[name of Group, Approach, or Technique], [name of mechanism question]	This Group/Approach/Technique was applied to answering this mechanism question.
Ion	Metal ions released from particles into the exposure medium are necessary for antibacterial activity
Contact	Close association between the ENM and cell is necessary for antibacterial activity
Internalization	Internalization of intact ENMs across an intact cell membrane contributes significantly to antibacterial activity
ROS	The production or accumulation of ROS, intracellularly or extracellularly, contributes significantly to antibacterial activity
Photoactivity	The presence of light is essential for antibacterial activity
Membrane	Damage to the cell wall or membrane contributes significantly to antibacterial activity
DNA	Damage to bacterial DNA contributes significantly to antibacterial activity
Protein	The binding or inactivation of intra- or extracellular proteins by ENMs or their dissolved components contributes significantly to antibacterial activity

Table S3. Full list of mechanism-targeted experimental Techniques, categorized according to Group and Approach as explained in Table 4 of the main text.

Group	Approach		Technique	Endpoint	Type of Analysis	Used For	References
1	1	1	NOM (e.g. humic acid) addition	difference in toxicity	(dependent on toxicity assay)	ion	[1, 2]
		2	Insoluble salt-forming agent addition, including orthophosphate, sulfide, sodium thiosulfate, sodium chloride	difference in toxicity	(dependent on toxicity assay)	ion, contact	[2-5]
		3	Chelator addition, including NAC, EDTA, bathocuproine, neocuproine	difference in toxicity	(dependent on toxicity assay)	ion, contact	[3, 6-14]
		4	Use NP concentration below toxic ion release threshold	difference in toxicity	(dependent on toxicity assay)	ROS	[15]
	2	5	Compare colloidal to immobilized NPs	difference in toxicity	(dependent on toxicity assay)	ion, contact, internalization	[2, 16-18]
		6	Compare to NP-free filtrate	difference in toxicity	(dependent on toxicity assay)	ion, contact	[1, 4, 19-27]
		7	Membrane barrier	difference in toxicity	(dependent on toxicity assay)	ion, contact	[28-30]
		8	Induce aggregation, including extracellular polymeric substance (EPS) addition	difference in toxicity	(dependent on toxicity assay)	ion, contact	[1, 30-32]
		9	Compare to inert NPs of same size and morphology	difference in toxicity	(dependent on toxicity assay)	contact	[33-37]
		10	Compare toxicity in aerobic and anaerobic environment	difference in toxicity	(dependent on toxicity assay)	ion, contact, ROS	[3, 38, 39]
	3	11	Cysteine (e.g. NAC) addition	difference in toxicity	(dependent on toxicity assay)	ROS	[21, 24, 35, 39-43]
		12	Ascorbic acid addition	difference in toxicity	(dependent on toxicity assay)	ROS	[41, 44, 45]
		13	Methanol addition	difference in toxicity	(dependent on toxicity assay)	ROS	[46, 47]
		14	Vitamin E addition	difference in toxicity	(dependent on toxicity assay)	ROS	[28]
		15	Mannitol addition	difference in toxicity	(dependent on toxicity assay)	ROS	[28]
		16	GSH addition	difference in toxicity	(dependent on toxicity assay)	ROS	[5, 28, 44]
		17	SOD addition	difference in toxicity	(dependent on toxicity assay)	ROS	[10, 24, 43, 46]
		18	CAT addition	difference in toxicity	(dependent on toxicity assay)	ROS	[10, 43, 48, 49]
		19	DMSO addition	difference in toxicity	(dependent on toxicity assay)	ROS	[43]

		20	t-butanol addition	difference in toxicity	(dependent on toxicity assay)	ROS	[48]
		21	Ammonium oxalate addition	difference in toxicity	(dependent on toxicity assay)	ROS	[48]
		22	BQ addition	difference in toxicity	(dependent on toxicity assay)	ROS	[48, 50]
		23	p-benzoic acid addition	difference in toxicity	(dependent on toxicity assay)	ROS	[51]
		24	IPA addition	difference in toxicity	(dependent on toxicity assay)	ROS	[50]
		25	EDTA-Fe	difference in toxicity	(dependent on toxicity assay)	ROS	[52]
		26	TEMPOL	difference in toxicity	(dependent on toxicity assay)	ROS	[52]
	4	27	NP pre-irradiation	difference in toxicity	(dependent on toxicity assay)	photoactivity	[53-56]
		28	Compare toxicity in light vs dark conditions	difference in toxicity	(dependent on toxicity assay)	photoactivity	[13, 46, 57-81]
	5	29	Add osmotic support	difference in toxicity	(dependent on toxicity assay)	membrane	[82]
	6	30	Soluble salt (e.g. AgNO ₃ , CuSO ₄ , ZnCl ₂)	difference in toxicity	(dependent on toxicity assay)	ion, contact	[6-8, 13, 20, 22, 28-31, 34-36, 52, 54, 55, 59-61, 71, 77, 83-130]
		31	Metal plate	difference in toxicity	(dependent on toxicity assay)	ion, contact	[2, 16]
		32	H ₂ O ₂	difference in toxicity	(dependent on toxicity assay)	ROS	[13, 21, 30, 125]
		33	Detergent/bacteriolytic agent	difference in toxicity	(dependent on toxicity assay)	membrane	[97, 131-133]
2	7	34	Recombinant bioluminescent reporter strain	various (incl. intracellular ROS species, bioavailable metal, DNA damage, membrane damage)	chemiluminometric	ion, ROS, membrane, DNA, protein	[7, 10, 27, 40, 72, 83, 90, 93, 94, 115, 124, 134-137]
		35	Single-gene deletion ("knockout") strain	difference in toxicity	(dependent on toxicity assay)	ion, ROS, DNA	[93, 115, 137, 138]
	8	36	Transcriptome analysis	quantity of RNA produced relative to control (indicates up- and down-regulation of relevant genes)	various, usually microarray or high-throughput sequencing	ion, contact, ROS, membrane, DNA, protein	[21, 26, 36, 40, 72, 75, 99, 118, 123, 138-151]

		37	Proteome analysis	quantity of protein produced relative to control (indicates up- and down-regulation of relevant genes)	various, usually mass spectrometry	ion, contact, ROS, membrane, DNA, protein	[11, 44, 71, 97, 101, 104, 128, 145, 147, 148, 152-155]
	9	38	NBT assay	Usually superoxide anion levels; inhibition of stain is also used as a measure for SOD activity	colorimetric	protein	[156]
		39	Ammonium molybdate assay	CAT activity	colorimetric	ROS, protein	[6, 11, 24, 44, 133, 156]
		40	NADPH/NaNO ₃ assay	GPX activity	colorimetric	ROS	[11]
		41	CDNB/GSH assay	GST activity	colorimetric	ROS, protein	[11, 156]
		42	Peroxidase activity assays, including pyrogallol and guaiacol	Peroxidase activity	colorimetric or fluorometric	ROS	[44, 157]
		43	DTNB assay	GR activity	colorimetric	ROS, protein	[13, 24, 158]
		44	NADH assay (with INT or resorufin)	Dehydrogenase activity	colorimetric or fluorometric	protein, ROS	[11, 107, 117, 126, 152, 156, 158-161]
		45	Lipase activity (Randox)	Lipase activity	colorimetric	protein	[107, 156]
		46	ONPG assay	GAL activity	colorimetric	protein	[52, 162]
		47	Phenol red urease assay	Urease activity	colorimetric	protein	[163]
		48	Acetylene reduction assay	Nitrogenase activity	GC	protein	[151]
		49	P-NPP assay	Alkaline phosphatase activity	colorimetric	protein	[14]
3	10	50	XAS analysis of metal in cell mass or supernatant	Local geometric and electronic structure of metal atoms	EXAFS and/or XANES	ion, contact, internalization, ROS	[91, 164-167]
		51	XPS analysis of NP/cell interface	Oxidation state of metal	XPS	photoactivity, contact, ROS	[63, 73]
		52	Speciation modeling	Metal speciation in supernatant	in silico	ion, contact	[167]
	11	53	FTIR of cellular fraction or extracellular polymeric substances (EPS)	Chemical changes in cellular components	FTIR	contact, membrane, protein, DNA	[47, 48, 63, 71, 82, 84, 116, 166, 168-174]
		54	Raman spectroscopy of cellular fraction	Chemical changes in cellular components	Raman spectroscopy	protein, DNA	[25, 63, 175]
		55	Peptidoglycan analysis	Chemical changes in cell wall components	GC/MS and CD spectroscopy	membrane	[176]

		56	TBA/MDA assay	Degree of lipid peroxidation	colorimetric or fluorometric	ROS, membrane	[5, 21, 24, 43, 52, 58, 66, 83, 111, 156, 172, 177-186]
		57	DNP assay	Protein carbonyl content (indicates protein oxidation)	colorimetric	ROS	[155, 177]
		58	RNA degradation assay	Changes in RNA lengths relative to control	gel electrophoresis	protein	[187]
		59	AOPP assay	AOPP formation (indicates protein oxidation)	UV-Vis	protein, ROS	[182, 188]
4	12	60	Reversability study (i.e. recovery of cell growth after removal of NPs from system) for cell-associated metal	Growth rate relative to control	(dependent on toxicity assay)	ion, contact	[122]
		61	Metal concentration in cellular fraction, including sucrose gradient centrifugation assay	Quantity of metal associated with cells	ICP-MS	ion, contact, internalization	[96, 98, 128, 151, 164, 189-192]
		62	Metal concentration in cell-free filtrate	Quantity of metal remaining in suspension	ICP-MS	contact, internalization	[193]
		63	NPs suspended in supernatant	Quantity of colloidal metal removed from suspension compared to pre-cell exposure	UV-Vis	contact	[29]
	13	64	SEM	Qualitative attributes of cell/NP interaction	Image of cell exterior	contact, membrane	[5, 25, 30, 32, 42, 43, 48, 50, 62, 70, 82, 107, 113, 129, 151, 161, 181, 183, 184, 187, 193-223]
		65	SEM with elemental mapping (EDX or synchrotron XFM)	Qualitative attributes of cell/NP interaction	Image of cell exterior with elemental mapping	contact, membrane	[126, 127, 171, 224-228]
		66	AFM	Qualitative attributes of cell/NP interaction	Image of cell surface	contact, membrane	[14, 17, 130, 136, 170, 173, 197, 229-231]

	14	67	TEM	Qualitative attributes of cell/NP interaction	Image of cell interior	membrane, contact, internalization, DNA	[12, 30, 44, 52, 61, 64, 68, 84, 93, 113, 119, 129, 132, 141, 150-152, 161, 165, 168, 172, 176, 184, 212, 232-248]
		68	TEM with elemental mapping (EDX or synchrotron XFM)	Qualitative attributes of cell/NP interaction	Image of cell interior with elemental mapping	contact, internalization, membrane	[10, 19, 47, 83, 96, 100, 104, 150, 171, 179, 224, 226, 227, 249-253]
	15	69	CLSM	Qualitative attributes of cell/NP interaction (for intrinsically fluorescent NPs)	fluorescence microscopy	contact, internalization, membrane, DNA	[80, 237, 254-257]
		70	Two-photon microscopy with ion-specific label	Relative quantity of free ions within cells	fluorescence microscopy	ion, contact, internalization	[96]
		71	DLE with PI stain	Association between NPs and dead cells (for NPs with DLE, e.g., ZnO)	fluorescence microscopy	contact	[96]
		72	Dark-field microscopy (may be equipped with HSI)	Relative strength of interactions between NPs and cell surfaces	light microscopy	contact, membrane	[96, 99, 192, 258]
		73	Fluorescence microscopy with protein labeling	Localization of target proteins within cells	fluorescence microscopy	contact	[105, 192]
		74	NP tracking with intrinsic fluorescence or fluorescent label (e.g. rhodamine B)	Localization of NPs within cells	fluorescence microscopy	contact, internalization	[105, 155, 206]
		75	Light scattering method for particle internalization	Ratio of forward- to side-scattered light (varies with cell granularity)	light microscopy	internalization	[13, 201, 241, 259]
5	16	76	ONPG hydrolysis assay	GAL leakage	colorimetric	membrane	[45, 155, 169, 184, 195, 208, 260]
		77	K ⁺ and/or Mg ²⁺ leakage	K ⁺ and/or Mg ⁺ in supernatant	AAS/AES or selective electrode	membrane	[28, 106, 122, 129, 145, 168, 184, 218, 245]
		78	Tetraphenylborate assay	K ⁺ in supernatant	colorimetric	membrane	[48]
		79	TPP ⁺ leakage	TPP ⁺ in supernatant	selective electrode	membrane	[106]

		80	Nucleic acid leakage	Nucleic acids in supernatant	UV-Vis	membrane	[9, 43, 45, 98, 133, 155, 169, 194, 205, 211, 225, 240, 255, 261-263]
		81	Sodium pyruvate assay	LDH in supernatant	UV-Vis	membrane	[87, 104, 171, 174, 179, 191, 256, 264-266]
		82	Lowry method	Protein in supernatant	colorimetric	membrane	[45, 159, 201, 257, 260]
		83	Bradford method	Protein in supernatant	colorimetric	membrane	[48, 50, 117, 133, 156, 160, 161, 169, 214, 218, 228, 242, 262, 264, 267-269]
		84	Miller method	Reducing sugar in supernatant	colorimetric	membrane	[117, 126, 156, 159, 160, 242, 262, 267-269]
		85	DNA release, including diphenylamine and PicoGreen assays	DNA in supernatant	fluorometric	membrane	[9, 32, 98, 217, 258]
		86	RNA gel electrophoresis	RNA in supernatant	gel electrophoresis	membrane	[184]
		87	P-NPP assay	Alkaline phosphatase in supernatant	colorimetric	membrane	[14, 130, 264]
		88	EMA qPCR assay	DNA in cells with compromised membranes	gel electrophoresis	membrane	[204]
	17	89	DNA ladder assay	Degree of gDNA fragmentation	gel electrophoresis	DNA, protein	[45, 52, 107, 163, 165, 177-180, 215, 218, 265, 270]
		90	UV-Vis assay	Degree of gDNA fragmentation	spectrophotometric	DNA	[188, 237]
		91	HPLC	Degree of DNA oxidation	HPLC	DNA	[182, 188]
		92	Viscosity assay	Viscosity of DNA solution (as a metric for mode of interaction with NPs)	rotational viscometer	DNA	[178]
	18	93	DCFH-DA (including variants such as CM-H2DCFDA, ab113851-DCFDA, H2DCFDA, DCF-DA)	Intracellular ROS	fluorometric	ROS	[5, 21, 22, 24, 32, 39, 42-44, 62, 64, 67-69, 75-77, 83, 87, 88, 96, 120, 128, 132, 142, 149, 151, 156, 157, 159, 171-174, 177, 179-183, 186, 187, 189, 190, 192, 194-196, 201, 206, 207, 211, 212, 216, 225, 239, 241, 252, 256, 257, 259, 265, 271-275]

		94	NBT assay	Usually superoxide anion levels; inhibition of stain is also used as a measure for SOD activity	colorimetric	ROS	[11, 24, 44, 46, 54, 98, 157, 171, 174, 178, 225, 256, 262, 276]
		95	Rhodamine dyes, including DHR6G and dihydrorhodamine 123	Intracellular ROS	fluorometric	ROS	[82, 181, 277, 278]
		96	Hydroxylamine assay (cells must be lysed)	Intracellular O ₂ -	fluorometric	ROS	[44]
		97	Propidium iodide (PI) stain	Membrane permeability (enters cells with compromised membranes)	fluorometric	membrane	[9, 12, 20, 22, 32, 43, 47, 63, 68, 77, 87, 91, 96, 108, 113, 114, 126, 129, 138, 151, 165, 166, 171, 173, 183, 187, 189, 196, 199, 205, 212, 216, 225, 241, 263, 270, 273, 274, 279, 280]
		98	Ethidium bromide (EtBr) stain	Membrane permeability (enters cells with compromised membranes and stains DNA)	fluorometric	membrane	[174]
		99	DiBAC4 stain	Membrane potential	fluorometric	membrane	[114, 126, 149]
		100	diSC3(5) assay for membrane potential	Membrane potential	fluorometric	membrane	[52, 97, 201, 209, 258]
		101	1-NPN	Outer membrane permeability	fluorometric	membrane	[9, 106, 155, 183, 194, 211]
		102	DPH membrane fluidity assay	Membrane fluidity	fluorometric	membrane	[132, 201, 218, 273]
		103	ANS probe	Membrane potential	fluorometric	membrane	[188]
		104	TUNEL assay	Degree of apoptosis	fluorometric	DNA	[149, 201, 216]
		105	DAPI stain	Stains DNA	fluorometric	internalization	[179, 201]
		106	Hoechst 33342	Stains DNA	fluorometric	internalization	[189]
		107	Annexin V	Degree of apoptosis	fluorometric	protein	[44, 149, 281]
		108	CaspACE FITC-VAD-FMK stain	Intracellular caspase-like protein (as a marker for apoptosis)	fluorometric	protein	[149]
		109	RedoxSensor assay	Reductase activity	fluorometric	ROS	[91, 165, 166]

	19	110	DTNB assay for cellular GSH	Quantity of disulfide-containing molecules	colorimetric	ROS, protein	[13, 156, 173, 179, 181]
		111	8-oxoguanine assay for oxidative DNA damage	Oxidative damage	LC-MS/MS	DNA	[5]
		112	Dissolved oxygen	Cell respiration	colorimetric	membrane, protein	[106]
		113	MTT	Cell respiration	colorimetric	protein	[158]
		114	TTC reduction	Dehydrogenase activity	colorimetric	protein	[66, 191]
		115	Luciferin/trichloroacetic (TCA) assay for ATP content	Cell respiration	chemiluminometric	protein	[1, 43, 52, 83, 97, 126, 156, 218, 238]
		116	AMO- and HAO-specific SOUR measurements	Cell respiration (following blockage of one or multiple SOUR-relevant enzymes)	SOUR	protein	[122]
		117	NAD ⁺ /NADH ratio	Cell respiration	colorimetric	protein	[5]
		118	Intracellular K ⁺ or Ca ²⁺	Cell respiration (as a reflection of membrane damage and metabolic disruption)	flame AES	protein	[52, 97, 149]
		119	EPS quantification	EPS production (as a measure of cell viability or metabolic activity)	colorimetric	protein	[166, 191, 225]
		120	OmpA immunoblot	Accumulation of precursor forms of membrane proteins	immunoblot	protein	[97]
		121	Phag-GFP expression assay	Degree of Phag-GFP expression relative to control	chemiluminometric	protein	[165, 166, 282]
		122	X-gal plating assay	Portion of population expressing beta-galactosidase relative to control	colony color	DNA	[197]
		123	SDS-PAGE for general protein damage	Changes in protein masses compared to control	gel electrophoresis	protein	[108]
	20	124	EPR/ESR	Quantity of ROS (species depends on spin trap used)	ESR	ROS	[46, 67, 71, 78, 83, 99, 148, 151, 172, 205, 216, 275, 283, 284]

	21	125	Ames test	Number of mutations caused by material	number of colonies	DNA	[194, 211, 285-287]
	22	126	EtBr cartwheel efflux pump inhibition assay	Eliminates ion efflux inhibition	Role of intracellular ions in toxicity and corresponding cellular defense mechanisms	ions, internalization	[9]
6	23	127	NP-protein interaction modeling	Number and type of possible interactions between NPs and target proteins	in silico	contact, protein, membrane	[288]
		128	NP/peptidoglycan interaction modeling	Theoretical affinity of NPs for peptidoglycan	in silico	contact	[253]
		129	in vitro analysis of fatty acids on NP thin film	Chemical and structural changes in fatty acids (key components of plasma membrane)	XPS	membrane	[70]
		130	in vitro peptidoglycan assay	Chemical changes in peptidoglycan (key component of cell wall)	UV-Vis	protein	[258]
		131	in vitro resorufin β -D-galactopyranoside assay	NP inhibition of GAL in vitro	fluorometric	protein	[162]
		132	in vitro enzyme activity assay	NP inhibition of enzyme activity in vitro	various, usually colorimetric	protein	[52, 87, 104]
		133	In vitro disulfide bond interaction assay	Amount of insulin bound to NPs	gel electrophoresis	protein	[107]
		134	In vitro protein binding assay	Amount of GAL binding to NPs	gel electrophoresis	protein	[162]
		135	In vitro protein binding assay	Amount of BSA bound to NPs	UV-Vis	protein	[260]
	24	136	in vitro DNA fragmentation assay	Degree of plasmid fragmentation	gel electrophoresis	DNA	[178, 189, 267]
		137	in vitro DNA interaction assay	Amount of DNA bound to NPs	UV-Vis	DNA	[177]
	25	139	pyranine lipid vesicle assay	Degree of synthetic membrane permeability	pH change	contact	[30, 49]
		140	liposome carboxyfluorescein assay	Degree of synthetic membrane permeability	fluorometric	membrane	[28, 231]

		141	Synthetic membrane surface pressure assay	Surface pressure on membrane (as a measure of mechanical stress created by NPs)	DLS	membrane	[289]
		142	Three-electrode assay with synthetic (e.g. DOPC) membrane	Electrostatic attraction between NPs and synthetic membrane	three-electrode system	contact	[219]
		143	in vitro TBA/MDA assay	Degree of lipid peroxidation in vitro	colorimetric or fluorometric	ROS	[55, 111]
	26	144	e.g. fumarase A and sulfite reductase			protein	[98]
7	27	145	ICP-MS, -OES, -AES	Concentration of ions in exposure media	ICP-MS, -OES, -AES	ion	[6, 8, 11-16, 19, 20, 34, 36, 38, 42, 43, 51, 58, 60, 61, 64, 71, 72, 77, 79, 82, 84, 94-97, 99, 101, 103, 108, 110-112, 114, 115, 120-122, 125, 127, 128, 130, 132, 142, 157, 164, 172-174, 178, 189, 193, 210, 216, 217, 226, 250, 252, 259, 261, 278, 279, 283, 284, 290-301]
		146	AAS	Concentration of ions in exposure media	AAS	ion	[10, 27, 29, 32, 46, 48, 54, 91, 113, 116, 124, 135, 138, 191, 199, 200, 205, 266, 302, 303]
		147	HPLC		HPLC	ion	[23]
		148	ASV	Concentration of ions in exposure media		ion	[304]
		149	chloride precipitation	Concentration of ions in exposure media	colorimetric	ion	[41]
		150	silver/sulfide electrode	Concentration of ions in exposure media	selective electrode	ion	[29, 43, 88, 277, 282]
		151	Alizarin red S (ARS)	Concentration of ions in exposure media	colorimetric	ion	[9]

	28	152	GSH oxidation (DTNB)	ROS quantity	colorimetric	ROS, protein	[19, 58, 174, 199, 207, 300]
		153	XTT	Superoxide quantity	colorimetric	ROS	[59-61, 79, 187, 194, 199, 211, 300, 305]
		154	HE (hydroethidene)	Superoxide quantity		ROS	[172]
		155	Methyl orange	ROS quantity		photoactivity, ROS	[172, 294, 295]
		156	Methylene blue	ROS quantity		photoactivity, ROS	[53, 68, 69, 146, 261, 306]
		157	3'-(p-aminophenyl) fluorescein (APF)	Hydroxyl quantity		ROS	[21, 88, 146]
		158	3-(p-hydroxyphenyl) fluorescein (HPF)			photoactivity, ROS	[47, 172]
		159	Rhodamine B			ROS	[244]
		160	KI UV-Vis assay for H2O2	H2O2 quantity	colorimetric	ROS	[278]
		161	DMSO H2O2 colorimetric assay	H2O2 quantity	colorimetric	ROS	[302]
		162	HRP horseradish peroxide assay	H2O2 quantity		ROS	[28]
		163	KMnO4 redox titration			ROS	[46, 276]
		164	DCPIP			photoactivity, ROS	[72]
		165	DPPH			ROS	[228]
		166	Terephthalic acid (or Phth)	Hydroxyl quantity		ROS	[15, 46, 50, 276]
		167	Luminol for superoxide	Superoxide quantity	chemiluminometric	ROS	[15, 83]
		168	Luminol+ferricyanide for H2O2	H2O2 quantity	chemiluminometric	ROS	[15]
		169	p-chlorobenzoic acid (pCBA)			ROS	[51, 59-61, 79]
		170	FFA			ROS	[59-61, 79]
		171	Amplex Red	H2O2 quantity	colorimetric	ROS	[49]
		172	Singlet Oxygen Sensor Green (SOSG)	Singlet oxygen quantity	fluorometric	ROS	[172]
		173	Pentafluorobenzenesulfonyl fluorescein (PFBSF)		fluorometric	ROS	[172]
		174	Deoxyribose			ROS	[98]
	29	175	Electrical impedance (for film-embedded NPs)	difference in toxicity	(dependent on toxicity assay)	contact	[307]
		176	Defect sites or oxygen vacancies (measured by PL spectroscopy or XPS)	difference in toxicity	(dependent on toxicity assay)	ROS	[146, 276, 299, 308-311]
		177	Morphology	difference in toxicity	(dependent on toxicity assay)	ions, contact	[270, 293, 300, 306, 312-315]

		178	Zeta potential	difference in toxicity	(dependent on toxicity assay)	contact, membrane	[14, 41, 65, 95, 130, 135, 136, 171, 173, 174, 190, 252, 295]
		179	Surface coating	difference in toxicity	(dependent on toxicity assay)	ion, contact	[33, 89, 170, 316, 317]
		180	Interaction energy (calculated based on EDLVO theory)	difference in toxicity	(dependent on toxicity assay)	ROS	[276]
30	181	model organism Gram type	difference in toxicity	(dependent on toxicity assay)	contact	[62, 221, 309]	

Table S4. List of abbreviations and acronyms used in Table 3 and Table S3

AOPP	Advanced oxidation protein products
CAT	catalase
CD	Circular dichroism
CDNB	1-chloro-2,4-dinitrobenzene
CLSM	confocal laser scanning microscopy
DAPI	4',6-diamidino-2-phenylindole
DCFH-DA	2',7'-Dichlorofluorescein Diacetate
DiBAC4(3)	Bis-(1,3-Dibutylbarbituric Acid)Trimethine Oxonol
DiSC3(5)	3,3'-diphenylthiocarbocyanine iodide
DLE	deep level emission
DMSO	dimethyl sulfoxide
DNPH	2,4-dinitrophenylhydrazine
DPH	Diphenylhexyltriene
DTNB	5,5'-dithiobis-(2-nitrobenzoic acid)
EDTA	Ethylenediaminetetraacetic acid
EMA	ethidium monoazide
EPR	electron paramagnetic resonance spectroscopy
EPS	extracellular polymeric substance
ESR	electron spin resonance spectroscopy
FFA	furfuryl alcohol
GAL	Beta-galactosidase
GC	gas chromatography
GFP	green fluorescent protein
GPX	Glutathione peroxidase
GR	glutathione reductase
GSH	glutathione
GST	glutathione-s-transferase
H-NS	histone-like nucleoid structuring proteins
INT	Iodonitrotetrazolium
IPA	isopropanol
LDH	lactate dehydrogenase
MDA	Malondialdehyde
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
Na ₂ S ₂ O ₃	sodium thiosulfate
NAC	N-acetyl L-cysteine
NaCl	sodium chloride
NADH	1,4-Dihyronicotinamide adenine dinucleotide
NADPH	beta-Nicotinamide Adenine Dinucleotide Phosphate, Reduced
NBT	Nitro blue tetrazolium

NOM	natural organic matter
ONPG	o-nitrophenyl-beta-D-galactopyranoside
P-NPP	para-nitrophenol phosphate
Pyranine	8-hydroxy-1,3,6-pyrene-trisulfonate
qPCR	quantitative polymerase chain reaction
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
SOD	superoxide dismutase
SOUR	specific oxygen uptake rate
TBA	Thiobarbituric acid
TEMPO	4-Hydroxy-TEMPO or 4-hydroxy-2,2,6,6-tetramethylpiperidin-1-oxyl
TPP	tetraphenylphosphonium
TTC	2,3,5-triphenyltetrazolium chloride
TUNEL	terminal deoxynucleotidyl transferase dUTP nick end labeling
XAS	X-ray absorption spectroscopy, including XANES (X-ray absorption near edge structure) and/or EXAFS (extended X-ray absorption fine structure)
XTT	2,3-Bis-(2-Methoxy-4-Nitro-5-Sulfophenyl)-2H-Tetrazolium-5-Carboxanilide

Table S5. Summary of advantages and limitations of specific method Groups, Approaches, and Techniques that have been proposed in the literature. When advantages and limitations were reported for multiple sets of methods containing a particular Technique, this Technique is listed multiple times. For definitions of specific Groups, Approaches, and Techniques, see Table 4 in the main text.

Group	Approach	Technique	Documented Advantages and/or Limitations
1	2	10	Dissolution may occur through non-oxidative means, so anaerobic conditions may not eliminate antibacterial activity from dissolved ions [318].
	3	12, 16	Enzymes that utilize GSH and ascorbate are not present in certain bacterial species including <i>E. coli</i> , so these compounds are not useful as “diagnostic antioxidants” [319].
	6	30	The kinetics of ion leaching from ENMs are not captured in pulse ion addition [99]. Exposure media: certain ligands may generate misleading results by preferentially decreasing the bioavailability and antibacterial activity of dissolved ions [3]. Dose of soluble salt: must deliver the dose that the bacteria actually receive on ENM exposure in the relevant study conditions, rather than simply matching the mass of salt to the mass of ENMs [318]. If salt dosing is based on ion leaching experiments, the conditions of these assays may also affect results (see Group 7). Antibacterial activity of the counter-ion in soluble salt experiments [28]: can be mitigated by removing the anion from the system or controlling for its toxic effect.
2	7	34	Recommended as a screening step for multiple mechanisms due to their relative simplicity [320]. Metal-sensing biosensors may be the most accurate way to assess the “true bioavailable content” [321]. Biosensors are not as precise as analytical methods for assessing cell-associated metal, and the “bioavailable” fraction excludes complexes which have been shown to exert toxic effects in bacteria [115].
	8	36, 37	Ability to detect “broad coordinated trends” [322], particularly useful for providing more detailed resolution of “cell effects” aspects of antibacterial activity mechanism (e.g., membrane and DNA damage) [104, 124]. High-throughput methods are reliant on precise and accurate understanding of the function of genes in the model organism, including reference genes whose expression is not expected to change during the study [36]. Since a complete picture of gene function and bacteria physiology is not straightforward to achieve, misinterpretations may lead to erroneous conclusions, similar to those revealed about the role of ROS in the action of several antibiotics [319]. Transcriptome analysis may require the establishment of a “normal” or “background” cell phenotype, which can vary between studies [323]. Protein expression varies depending on stage of cell cycle, which introduces added variability into proteome analysis results [323].

3	(All Group 3)		Ability to connect the binary presence or absence of a mechanistic phenomenon to either its origin (i.e. ions or particles) or the chemical changes responsible. Most Group 3 methods are readily conducted at multiple time points to study the kinetics of cellular degradation [324].
	10	50	XPS and XAS of cellular metal can help establish whether intracellular metal represented in-tact ENMs or re-oxidized ions [91, 165, 166] .
	11	53	FTIR promoted as a means to show specific chemical changes that take place during membrane and protein damage [324].
4	12	61	Metal partitioning studies are the only quantitative means to assess cell-associated metal, but contain no information about ENM localization or whether the metal in question represents particles or dissolved ions [325].
	13, 14	64, 65, 67, 68	Protocols to minimize TEM and SEM artefacts are outlined in [326]. Potential for artefacts with TEM and SEM [327], including dark spots from osmium tetroxide residue that may be mistaken for ENMs [16, 326], as well as less readily identifiable artefacts that arise from the reaction of ENMs with fixation or staining agents [326]. TEM is known to induce "slight membrane damage" during sample preparation [166]. Lack of z-resolution in TEM may make it unsuitable for studying internalization [36]. ENMs may detach from membranes during sample preparation, generating false negatives in investigations of contact-mediated antibacterial activity mechanisms [96].
	14	66	AFM is adept at detailed study of cell surfaces and their mechanical properties [324], for example, through calculation of surface roughness and force-distance curves and the use of chemical force mapping [328]. Some have touted AFM as a means to move beyond simple binary designations of cell-ENM interactions (e.g., "morphology change" and "membrane damage") and towards a more complex understanding of the mechanical changes that occur in the cell as a result of ENM exposure [329].
	15	(All Approach 15)	High-throughput assays using fluorescence or light-scattering involve no fixation procedure and may help discern between ENM internalization and adsorption to the outer cell surface at the "bulk" level [325, 330]. These methods also enable the correlation of internalization rate with antibacterial activity in order to establish whether NP internalization is actually important [96, 259]. Lower resolution of high-throughput methods leave them powerless to discern ENM localization in detail, and they must be coupled with ICP-MS or other analytical method to provide quantitative information [325]. Light-scattering methods were adapted from eukaryotic cells and are comparatively less well-tested in prokaryotes [13].

	(All Group 5)		The majority of methods are fluorescent probes which, while commonly used, are not recommended as the sole means of gauging ROS accumulation; ESR/EPR (Approach 20, Technique 134) has been proposed as an alternative [331].
	16	81	Purportedly not suitable for ENMs that bind proteins or produce ROS [332], which potentially includes all the ENM classes reviewed here, since they may inhibit the enzymatic conversion by LDH. Further ambiguities are created if the material absorbs at the necessary wavelength, which may include nAg [333].
5	18	(All Approach 18)	Intracellular probes may penetrate cells to differing degrees depending on membrane permeability [132], may be actively excreted by bacterial pmf-dependent pumps [319], and may artificially appear to fluoresce more due to the morphological changes that take place in bacteria under stress [319].
		93	DCFH-DA may be subject to autocatalytic degradation [323], and different preparation protocols have been shown to yield different results depending on the time at which the probe is added, the incubation time, and the predominant species [334].
		93, 94, 95	Dyes which operate via a radical intermediate, including fluorescein and rhodamine dyes, NBT, and lucigenin, may be sensitive to redox-cycling mechanisms in the presence of oxygen that artificially amplify the fluorescence signal [319, 331, 335].
		97, 105, 106	The PI, DAPI, and Hoechst stains are subject to background signal from ENMs with red or blue fluorescence [333].
	21	125	The Ames reverse mutation test, a staple of the genotoxicity testing toolkit, is shown by multiple sources to be inappropriate for studies of DNA damage in ENMs due to its tendency to cause premature negative conclusions. Reasoning ranges from the high antibacterial activity of ENMs and their tendency to cause large-scale chromosomal damage as opposed to point mutations [194, 336] to the potentially limited uptake of ENMs into the cytoplasm [337] and the aggregation and lack of electrostatic attraction between ENMs and bacteria in the standard test medium [287].
7	27	(All Approach 27)	For ion release studies, the extent of dissolution depends heavily on media composition [23, 338, 339] and presence or absence of cells [115, 339]. Important variables to control for in exposure media include pH, ionic strength, NOM, and oxygen content [340]. Dissolution often takes place at the nano-bio interface, and specific proteins found in bacteria have been found to mediate oxidative dissolution while others do not, an example of complex dissolution processes which are not captured in acellular dissolution assays [341]. Measuring the concentration of dissolved ions does not account for the fraction of ions that are bioavailable, nor does it establish conclusively that the ions actually contribute to antibacterial activity [342].

	28	(All Approach 28)	Extracellular ROS measurements were found to be affected by pyruvate, a common ingredient in cell media that acts as an ROS scavenger [343]. Tetrazolium derivatives may be adsorbed to ENMs and lose activity [333].
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Table S6. Mean and standard deviation of bootstrapped Consistency Scores, disaggregated by core composition. The number of studies in each set is also listed in the “n” columns.

Question	Group	Approach	Technique	Ag			Cu			CuO			TiO2			ZnO		
				Median	StDev	n	Median	StDev	n	Median	StDev	n	Median	StDev	n	Median	StDev	n
Ion	(all)			0.143	0.050	55	0.163	0.117	9	0.122	0.078	19	0.500	0.000	0	0.055	0.037	31
	1			0.107	0.062	27	0.280	0.171	3	0.100	0.072	12	0.500	0.000	0	0.078	0.052	14
	1	1		0.168	0.120	5	0.500	0.000	2	0.500	0.000	2	0.000	0.000	0	0.500	0.000	1
	1	1	3	0.275	0.179	3	0.000	0.000	0	0.186	0.161	2	0.000	0.000	0	0.208	0.154	2
	1	2		0.218	0.124	6	0.000	0.000	0	0.223	0.151	2	0.000	0.000	0	0.229	0.143	2
	1	2	6	0.132	0.070	21	0.262	0.251	1	0.146	0.097	12	0.500	0.000	0	0.074	0.061	12
	1	6		0.107	0.070	21	0.243	0.251	1	0.167	0.084	12	0.500	0.000	0	0.083	0.056	12
	1	6	30	0.128	0.069	21	0.213	0.248	1	0.155	0.103	12	0.500	0.000	0	0.083	0.057	12
	2			0.313	0.113	9	0.500	0.000	0	0.500	0.000	2	0.500	0.000	0	0.173	0.116	3
	2	7		0.386	0.109	7	0.000	0.000	0	0.500	0.000	2	0.500	0.000	0	0.270	0.179	3
	2	7	34	0.357	0.125	6	0.000	0.000	0	0.500	0.000	2	0.500	0.000	0	0.270	0.186	3
	4			0.358	0.166	2	0.266	0.153	2	0.299	0.164	1	0.000	0.000	0	0.500	0.000	0
	4	12		0.342	0.167	2	0.269	0.155	2	0.279	0.158	1	0.000	0.000	0	0.500	0.000	0
	7			0.146	0.066	34	0.190	0.116	6	0.186	0.108	10	0.500	0.000	0	0.081	0.065	24
	7	27		0.131	0.069	33	0.197	0.124	6	0.167	0.097	10	0.500	0.000	0	0.089	0.057	24
	7	27	145	0.131	0.079	23	0.292	0.181	1	0.214	0.113	9	0.500	0.000	0	0.117	0.072	20
7	27	146	0.260	0.132	6	0.500	0.000	4	0.238	0.251	1	0.000	0.000	0	0.156	0.126	3	
Particle	(all)			0.338	0.043	71	0.333	0.091	14	0.317	0.090	18	0.392	0.074	17	0.332	0.053	41
	1			0.288	0.067	22	0.292	0.162	2	0.258	0.127	9	0.000	0.000	0	0.243	0.128	8
	1	2		0.350	0.101	10	0.000	0.000	0	0.314	0.153	4	0.500	0.000	0	0.500	0.000	0
	1	6		0.323	0.069	17	0.500	0.000	2	0.211	0.122	6	0.000	0.000	0	0.285	0.119	8
	1	6	30	0.303	0.086	16	0.500	0.000	2	0.194	0.116	6	0.000	0.000	0	0.287	0.128	8
	3			0.260	0.187	3	0.000	0.000	0	0.500	0.000	0	0.500	0.000	4	0.500	0.000	3
	4			0.385	0.047	44	0.324	0.112	9	0.500	0.000	13	0.376	0.075	14	0.368	0.072	20
	4	12		0.275	0.185	3	0.272	0.156	2	0.500	0.000	2	0.500	0.000	1	0.500	0.000	1

	4	13		0.452	0.054	19	0.500	0.000	3	0.500	0.000	6	0.347	0.131	6	0.362	0.092	13
	4	13	64	0.500	0.000	11	0.500	0.000	2	0.500	0.000	5	0.337	0.149	5	0.344	0.110	10
	4	14		0.359	0.059	30	0.318	0.150	4	0.500	0.000	5	0.392	0.116	7	0.402	0.084	9
	4	14	67	0.369	0.061	24	0.500	0.000	3	0.500	0.000	2	0.500	0.000	5	0.338	0.142	5
	4	14	68	0.361	0.125	6	0.243	0.251	1	0.500	0.000	3	0.276	0.157	2	0.500	0.000	4
	7			0.373	0.121	7	0.500	0.000	2	0.500	0.000	1	0.500	0.000	2	0.314	0.155	5
	7	29		0.378	0.125	6	0.500	0.000	2	0.500	0.000	1	0.500	0.000	2	0.314	0.145	4
	7	29	178	0.292	0.187	3	0.500	0.000	1	0.500	0.000	1	0.500	0.000	2	0.500	0.000	2
Internalization	(all)			0.273	0.061	35	0.393	0.104	8	0.166	0.105	5	0.500	0.000	7	0.198	0.096	14
	4			0.237	0.080	23	0.500	0.000	6	0.290	0.153	4	0.500	0.000	6	0.246	0.119	8
	4	14		0.189	0.096	15	0.500	0.000	3	0.285	0.173	3	0.500	0.000	6	0.286	0.123	8
	4	14	67	0.133	0.083	13	0.500	0.000	2	0.500	0.000	1	0.500	0.000	5	0.265	0.173	3
	4	14	68	0.500	0.000	5	0.500	0.000	1	0.276	0.157	2	0.500	0.000	1	0.342	0.150	5
ROS	(all)			0.232	0.057	53	0.500	0.000	8	0.246	0.078	19	0.231	0.075	27	0.306	0.046	55
	1			0.394	0.096	10	0.500	0.000	2	0.000	0.000	0	0.500	0.000	2	0.262	0.117	9
	1	3		0.500	0.000	8	0.500	0.000	2	0.000	0.000	0	0.500	0.000	1	0.276	0.132	7
	2			0.221	0.094	16	0.500	0.000	2	0.315	0.167	2	0.199	0.125	3	0.272	0.191	3
	2	7		0.201	0.125	3	0.500	0.000	2	0.500	0.000	2	0.000	0.000	0	0.500	0.000	2
	2	8		0.248	0.123	8	0.000	0.000	0	0.500	0.000	0	0.188	0.115	3	0.240	0.197	3
	2	8	36	0.189	0.124	6	0.000	0.000	0	0.500	0.000	0	0.265	0.135	2	0.500	0.000	2
	3			0.500	0.000	7	0.500	0.000	3	0.500	0.000	3	0.348	0.144	5	0.251	0.148	5
	3	11		0.500	0.000	7	0.500	0.000	3	0.500	0.000	2	0.500	0.000	4	0.233	0.137	5
	3	11	56	0.500	0.000	6	0.500	0.000	3	0.500	0.000	2	0.500	0.000	4	0.195	0.145	4
	5			0.251	0.065	33	0.500	0.000	3	0.211	0.107	9	0.215	0.107	9	0.203	0.086	20
	5	18		0.248	0.067	32	0.500	0.000	3	0.229	0.112	8	0.301	0.120	8	0.237	0.090	17
	5	18	93	0.255	0.064	29	0.500	0.000	2	0.202	0.118	6	0.308	0.116	8	0.236	0.098	11
	5	18	94	0.368	0.124	6	0.500	0.000	1	0.500	0.000	1	0.500	0.000	2	0.500	0.000	5
	5	20		0.500	0.000	2	0.490	0.070	0	0.282	0.172	3	0.285	0.160	1	0.163	0.126	5
	5	20	124	0.500	0.000	2	0.000	0.000	0	0.272	0.187	3	0.243	0.140	1	0.165	0.135	5
7			0.500	0.000	5	0.000	0.000	0	0.191	0.170	2	0.500	0.000	6	0.354	0.073	24	

	7	28		0.500	0.000	5	0.490	0.070	0	0.200	0.162	2	0.500	0.000	6	0.250	0.117	9
	7	28	153	0.500	0.000	3	0.000	0.000	0	0.500	0.000	0	0.500	0.000	1	0.338	0.146	5
Photoactivity	(all)			0.373	0.115	7	0.500	0.000	1	0.272	0.187	3	0.139	0.108	7	0.230	0.105	14
	1			0.344	0.126	6	0.500	0.000	1	0.280	0.171	3	0.119	0.105	5	0.237	0.099	11
	1	4		0.364	0.118	6	0.500	0.000	1	0.282	0.183	3	0.128	0.102	5	0.242	0.106	11
	1	4	28	0.385	0.128	6	0.000	0.000	0	0.250	0.170	3	0.127	0.099	5	0.171	0.115	7
	(all)			0.448	0.021	91	0.500	0.000	14	0.377	0.076	18	0.415	0.058	22	0.453	0.030	42
Membrane	2			0.500	0.000	7	0.500	0.000	2	0.500	0.000	1	0.500	0.000	7	0.500	0.000	4
	2	8		0.500	0.000	6	0.500	0.000	1	0.500	0.000	1	0.500	0.000	7	0.500	0.000	4
	2	8	36	0.500	0.000	3	0.500	0.000	1	0.500	0.000	1	0.500	0.000	5	0.000	0.000	0
	2	8	37	0.500	0.000	3	0.000	0.000	0	0.000	0.000	0	0.500	0.000	4	0.500	0.000	4
	3			0.500	0.000	11	0.500	0.000	2	0.500	0.000	1	0.500	0.000	6	0.500	0.000	10
	3	11		0.500	0.000	11	0.500	0.000	2	0.500	0.000	1	0.500	0.000	6	0.500	0.000	10
	3	11	53	0.500	0.000	3	0.000	0.000	0	0.000	0.000	0	0.500	0.000	4	0.500	0.000	8
	3	11	56	0.500	0.000	7	0.500	0.000	2	0.500	0.000	1	0.500	0.000	4	0.500	0.000	5
	4			0.482	0.019	52	0.500	0.000	5	0.406	0.074	10	0.301	0.126	8	0.464	0.037	26
	4	13		0.466	0.034	27	0.500	0.000	2	0.354	0.116	6	0.500	0.000	5	0.500	0.000	17
	4	13	64	0.500	0.000	18	0.500	0.000	1	0.500	0.000	6	0.500	0.000	5	0.500	0.000	14
	4	14		0.466	0.030	30	0.500	0.000	3	0.500	0.000	4	0.280	0.188	3	0.424	0.069	13
	4	14	67	0.464	0.034	26	0.500	0.000	2	0.500	0.000	3	0.275	0.175	3	0.399	0.099	9
	4	14	68	0.500	0.000	4	0.500	0.000	1	0.500	0.000	1	0.000	0.000	0	0.500	0.000	4
	5			0.440	0.032	47	0.500	0.000	6	0.325	0.120	9	0.393	0.089	10	0.500	0.000	17
	5	16		0.441	0.043	29	0.500	0.000	4	0.328	0.154	5	0.381	0.106	8	0.500	0.000	11
	5	16	80	0.393	0.099	9	0.500	0.000	1	0.500	0.000	1	0.500	0.000	2	0.500	0.000	3
	5	16	81	0.285	0.160	2	0.480	0.098	2	0.500	0.000	1	0.500	0.000	3	0.500	0.000	3
	5	16	83	0.500	0.000	12	0.000	0.000	0	0.000	0.000	0	0.500	0.000	1	0.500	0.000	4
	5	16	84	0.500	0.000	9	0.000	0.000	0	0.000	0.000	0	0.000	0.000	0	0.500	0.000	1
5	18		0.429	0.049	27	0.500	0.000	3	0.353	0.142	6	0.345	0.146	5	0.500	0.000	8	
5	18	97	0.402	0.066	18	0.500	0.000	2	0.368	0.119	6	0.500	0.000	2	0.500	0.000	6	
DNA	(all)			0.274	0.080	24	0.500	0.000	6	0.205	0.164	2	0.251	0.138	5	0.141	0.097	4

	2			0.357	0.131	6	0.500	0.000	2	0.500	0.000	0	0.295	0.182	3	0.500	0.000	0
	5			0.202	0.120	9	0.500	0.000	3	0.500	0.000	2	0.248	0.251	1	0.199	0.132	2
	5	17		0.283	0.145	6	0.500	0.000	3	0.500	0.000	1	0.000	0.000	0	0.275	0.195	1
	5	17	89	0.211	0.137	3	0.500	0.000	3	0.500	0.000	1	0.000	0.000	0	0.260	0.180	1
Protein	(all)			0.453	0.030	43	0.500	0.000	7	0.223	0.143	5	0.219	0.133	7	0.433	0.071	15
	2			0.462	0.040	22	0.500	0.000	3	0.294	0.151	4	0.170	0.119	4	0.395	0.111	8
	2	8		0.425	0.069	13	0.000	0.000	0	0.330	0.145	4	0.173	0.135	3	0.325	0.166	5
	2	8	36	0.401	0.109	8	0.000	0.000	0	0.297	0.172	3	0.155	0.132	3	0.304	0.160	4
	2	8	37	0.500	0.000	6	0.000	0.000	0	0.500	0.000	1	0.252	0.251	1	0.500	0.000	1

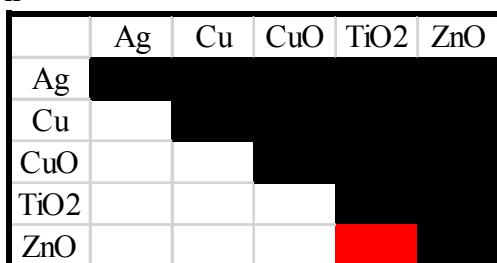
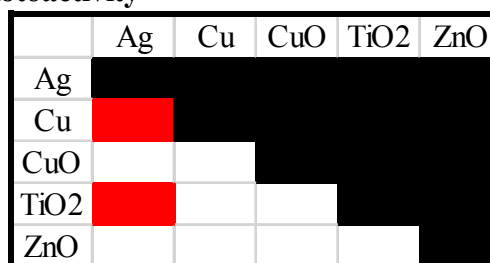
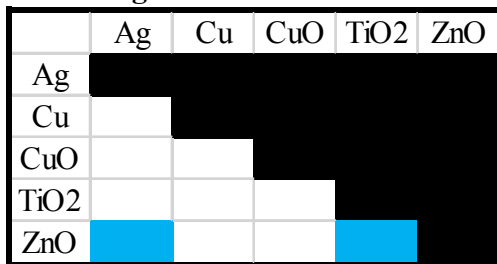
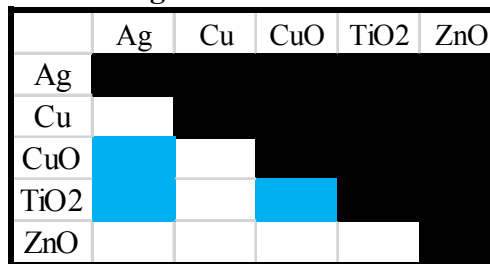
a. Ion**b. Photoactivity****c. DNA Damage****d. Protein Damage**

Figure S1. Statistically significant differences in conclusions based on ENM composition. Parts (a) through (d) show mechanism questions for which there were differences in conclusions between ENM compositions: ions (a), photoactivity (b), DNA damage (c), and protein damage (d). A blue cell indicates that the fraction of positive conclusions about the corresponding mechanism question was higher for the ENM composition on the horizontal axis versus the ENM composition on the vertical axis. A red cell indicates that the fraction of negative conclusions about the corresponding mechanism question was higher for the ENM composition on the horizontal axis versus the ENM composition on the vertical axis.

a. ENM particle size (dry, as measured by TEM)

	<10nm	10-20nm	20-30nm	30-40nm	40-50nm	>100nm	Compare multiple	Unspecified
Ion								ZnO
Particle				Ag		ZnO		
Internalization								
ROS			TiO2	TiO2	CuO		Ag	
Photoactivity	TiO2							
Membrane Damage								
DNA Damage	ZnO		ZnO					
Protein Damage								

b. ENM capping agent

	Uncapped	Unspecified
Ion		Cu
Particle		ZnO
Internalization		
ROS	TiO2	
Photoactivity		
Membrane Damage		
DNA Damage		
Protein Damage		

c. Bacterium used

	Gram positive	Gram negative	Both	Not specified
Ion				
Particle	ZnO	ZnO		
Internalization				
ROS			CuO	
Photoactivity		ZnO		
Membrane Damage		CuO, ZnO		
DNA Damage				
Protein Damage				

d. Characterization techniques

	SEM/TEM	DLS	Zeta potential	XRD	BET	UV-Vis	EDX	XPS	FTIR	PL Spectroscopy
Ion	ZnO				ZnO	Ag				ZnO
Particle	Cu					Ag				
Internalization										ZnO
ROS	TiO2	CuO TiO2		Ag, TiO2	ZnO	TiO2	Ag	Ag		
Photoactivity					TiO2					
Membrane Damage										
DNA Damage			Ag						ZnO	
Protein Damage	TiO2									

e. Antibacterial activity assessment method

	Plating	Disk diffusion	Broth dilution	Membrane	Other	Multiple concentrations	Multiple time points
Ion			ZnO	Ag	Ag		
Particle							
Internalization			Ag				Ag
ROS							ZnO
Photoactivity							
Membrane Damage							
DNA Damage	ZnO					Ag	
Protein Damage							TiO2

f. Lighting conditions (used in mechanism-targeted experiments)

	Dark	Unspecified
Ion	Ag ZnO	
Particle		
Internalization		ZnO
ROS		Ag
Photoactivity		
Membrane Damage		
DNA Damage		
Protein Damage	TiO2	

g. ENM hydrodynamic size (as measured by DLS)

	100-200nm	200-500nm	Compare multiple	Unspecified
Ion	ZnO		ZnO	
Particle				
Internalization				
ROS				ZnO
Photoactivity				
Membrane Damage				
DNA Damage				
Protein Damage				

h. Group number of mechanism-targeted experimental method (for explanation of method Groups, see Table S3 or Table 3)

	1	2	3	4	7
Ion		Ag			
Particle			CuO	ZnO	
Internalization					
ROS	Ag	TiO2			
Photoactivity					
Membrane Damage				Ag	
DNA Damage		Ag, TiO2			
Protein Damage					

Figure S2. Statistically significant differences in conclusions arising from selected study design variables, with different categories of study design variables (listed in Table 2 and Table S2) shown in parts (a) through (h). The eight mechanism questions are shown on the

vertical axis, and study design variables within each category are shown on the horizontal axis. Only variables which yielded a statistically significant difference in conclusions for at least one mechanism question, within at least one ENM composition, are included here; therefore, not all variables and categories listed in Table S2 are included. A blue cell indicates that the fraction of positive conclusions about the corresponding mechanism question was higher in the subset of studies for which the corresponding study design variable was true versus the subset of studies for which the corresponding study design variable was false, within the ENM composition group(s) specified. A red cell indicates that the fraction of negative conclusions about the corresponding mechanism question was higher in the subset of studies for which the corresponding study design variable was true versus the subset of studies for which the corresponding study design variable was false, within the ENM composition group(s) specified. A blank cell indicates that no statistically significant difference in conclusions was found for the corresponding mechanism question based on the corresponding study design variable, within any ENM composition group.

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