

Supporting Information

Antibacterial Effects of Carbon Nanotubes: Size Does Matter!

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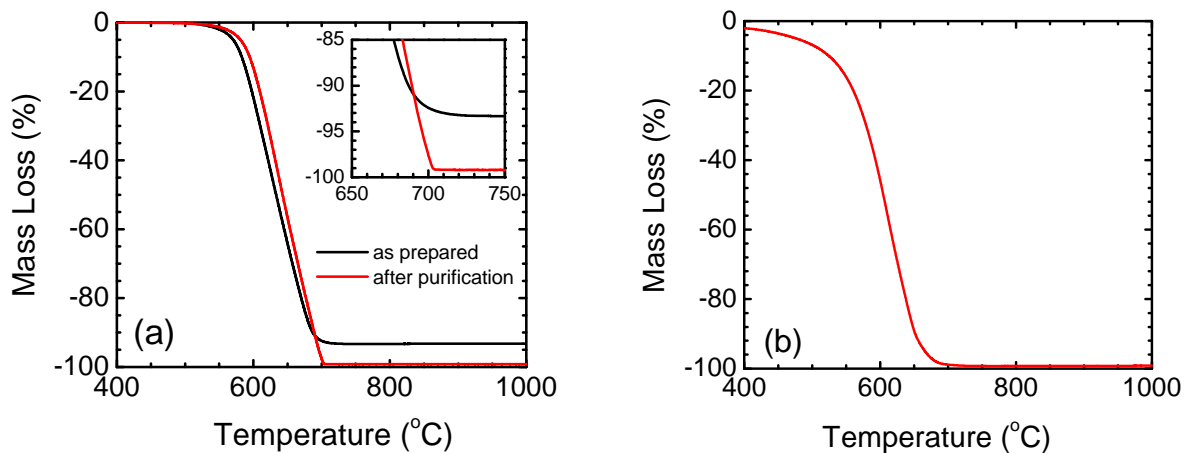


Figure S1. Mass loss curves from thermo-gravimetric analysis (TGA) of (a) MWNTs and (b) SWNTs. During the experiments, temperature was increased from 200°C to 1000°C at a rate of 10°C/min.

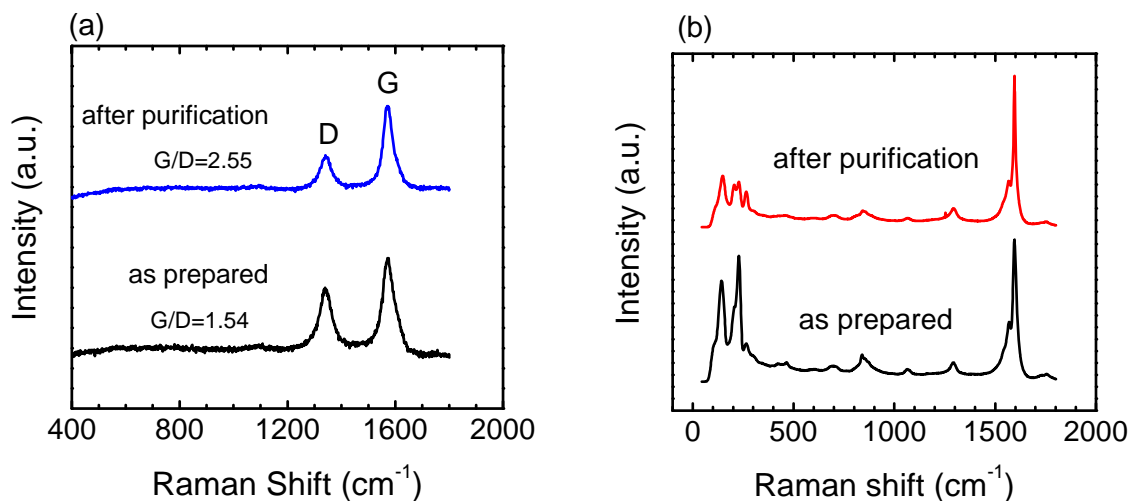


Figure S2. Raman spectra of carbon nanotubes before and after the purification steps: (a) MWNTs at 532 nm, (b) SWNTs at 785 nm

The most important feature in Figure S2a is the same position and width of the bands during our purification protocol. In addition, the intensity of the D-band (disorder band, peak at 1342 cm^{-1}) was reduced after the treatment with dry oxidation and non-oxidizing acid, while that of the G-band (graphite band, peak at 1572 cm^{-1}) was not changed in Figure S2a. We interpret the above Raman spectra as evidence for structural healing and effective metal removal in the MWNTs as a result of purification¹. Raman spectra also demonstrated that the whole purification procedure did not produce significant defects in the SWNTs (the G/D band ratio is essentially unchanged), as well as that the SWNT structure is well-preserved (as evidenced by the Raman breathing mode) (Figure S2b).

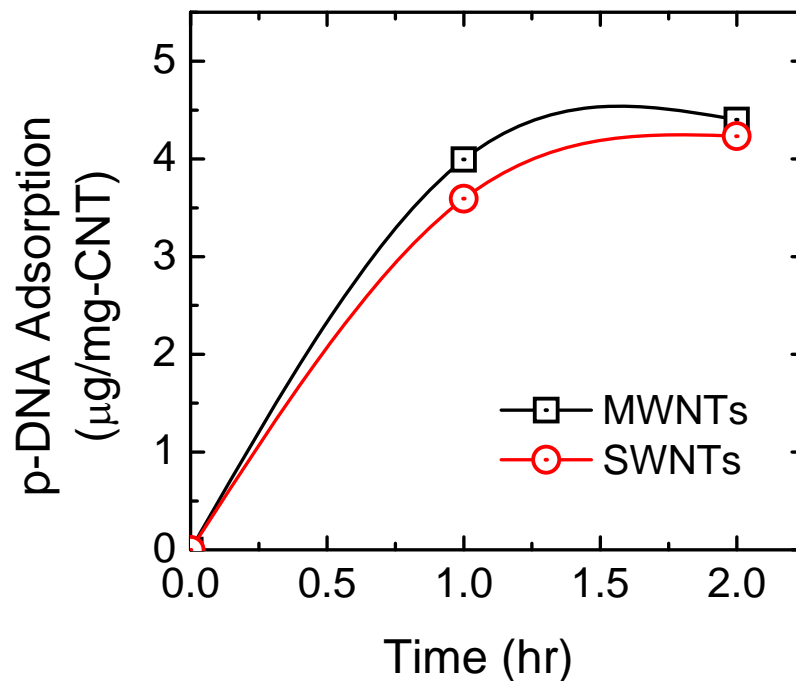


Figure S3. Plasmid DNA (p-DNA) adsorption capacity of MWNTs and SWNTs in 0.9 % (0.154 M) NaCl solution.

Method: p-DNA was extracted and purified with Qiagen plasmid kits (Midi25, Cat #121143). About 130 ng/mL (final concentration) of p-DNA was added into a glass bottle containing 50 mL (final volume) of 0.9 % (0.154 M) NaCl solution with 5 mg/L of either MWNTs or SWNTs (final concentration). Another sample under the same conditions but without CNTs was used as a control. Bottles were placed in a shaker (200 rpm) and incubated for 1 hr or 2 hr at 37°C. After incubation, the solution was filtered through a 0.22-µm low protein binding Millex membrane (Millipore) and the concentration of p-DNA in the filtrate was analyzed by fluorescence spectroscopy (Horiba, Japan) using DAPI as a fluorescent dye (excitation 370, emission 470 nm). Salmon sperm DNA (Invitrogen) was used as a standard (0 - 168 ng/mL).

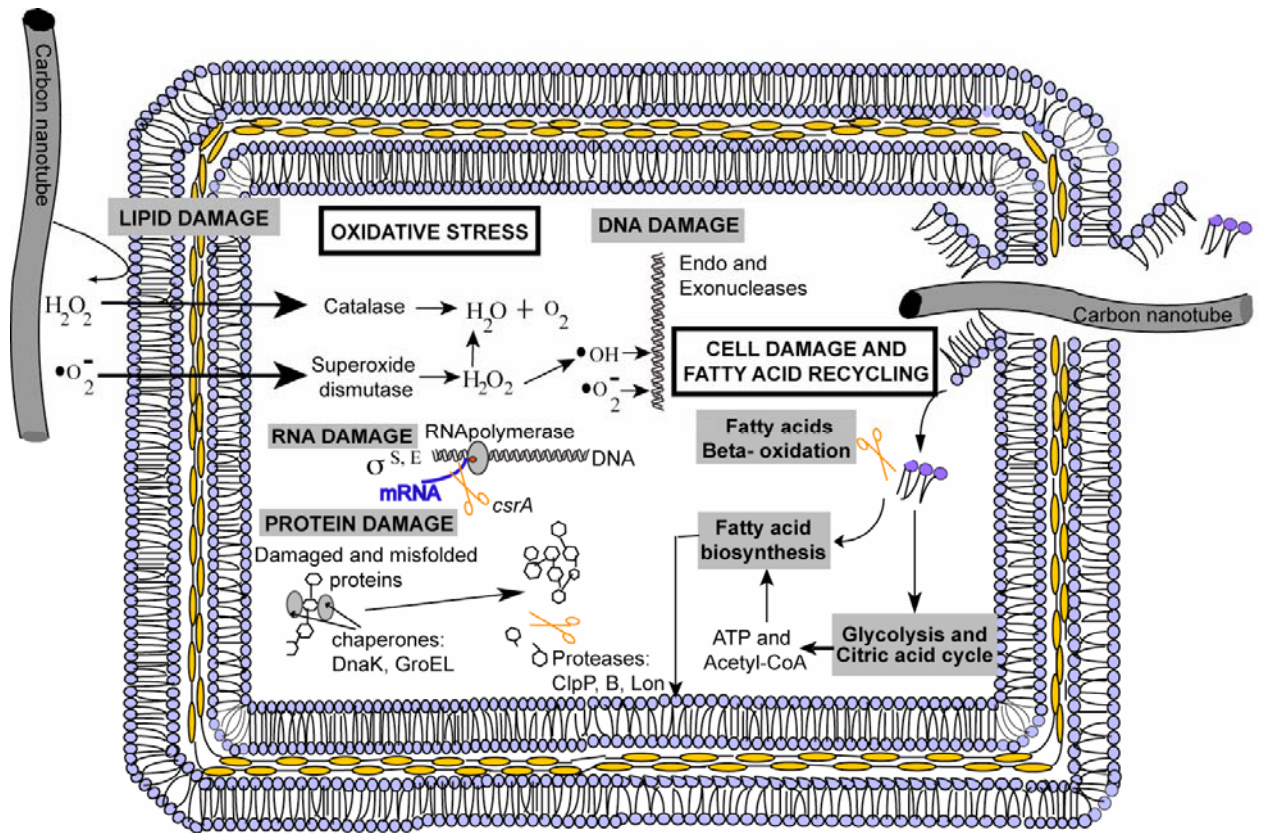


Figure S4. Schematic summary of *E. coli* K12 gene expression stress responses under exposure to SWNTs and MWNTs.

Table S1. Gene expression related to oxidative stress

Gene	b no.	SWNT Fold Change	MWNT Fold Change	Gene Function
OxyR regulon				
<i>ahpC</i>	b0605	3.25	2.46	Alkyl hydroperoxide reductase small subunit ^a
<i>dps</i>	b0812	3.25	3.25	Stress response DNA binding protein ^a
<i>fhuF</i>	b4367	2.64	2.00	Function Unknown ^a
<i>fur</i>	b0683	2.46	2.00	Negative regulator ^a
<i>gor</i>	b3500	1.52	Nd	glutathione oxidoreductase ^a
<i>yljA</i>	b0881	2.83	2.64	Function Unknown ^a
<i>katG</i>	b3942	1.52	1.52	Catalase hydrogen peroxidase I ^a
<i>sufA</i>	b1684	3.48	2.46	Homology with IscA ^a
<i>sufB</i>	b1683	3.03	2.30	Function Unknown ^a
<i>sufC</i>	b1682	3.03	1.62	Putative ABC transporter ^a
<i>sufD</i>	b1681	3.03	2.00	Function Unknown ^a
<i>sufE</i>	b1679	1.41	Nd	Function Unknown ^a
<i>sufS</i>	b1680	2.64	Nd	selenocysteine lyase ^a
<i>yaiA</i>	b0389	3.48	2.30	Function Unknown ^a
SoxRS regulon				
<i>acrA</i>	b0463	2.64	1.62	AcrAB efflux system effects Mar multiple resistance ^b
<i>acnA</i>	b1276	4.00	3.03	aconitate hydratase 1 ^b
<i>sodA</i>	b3908	3.73	2.83	superoxide dismutase, manganese ^b
<i>sodC</i>	b1646	3.73	3.03	superoxide dismutase precursor (Cu-Zn)
<i>zwf</i>	b1852	1.52	Nd	Glucose-6-phosphate dehydrogenase ^{b, c}
<i>fumC</i>	b1611	1.87	1.62	fumarate hydratase Class II; isozyme
<i>fldA</i>	b0684	1.52	Nd	flavodoxin 1
<i>tolC</i>	b3035	2.83	2.30	outer membrane channel; specific tolerance to colicin E1; segregation of daughter chromosomes
Miscellaneous genes related to oxidative stress				
<i>yfiA</i>	b2597	3.73	3.48	Function Unknown ^a
<i>cysK</i>	b2414	3.48	2.83	cysteine synthetase ^a
<i>ycgZ</i>	b1164	2.30	2.83	Function Unknown ^a
<i>ymgB</i>	b1166	2.64	3.03	Function Unknown ^a
<i>aldA</i>	b1415	5.28	2.30	Aldehyde dehydrogenase, NAD linked ^b
<i>artI</i>	b0863	3.48	2.14	Periplasmic binding protein of Arg transport system ^b
<i>artP</i>	b0864	2.83	2.46	Arg periplasmic transport system ^b
<i>ydbK</i>	b1378	3.25	2.14	putative pyruvate-flavodoxin oxidoreductase ^b
<i>cysD</i>	b2752	2.00	2.00	Sulfate Adenylyltransferase ^b
<i>dadX</i>	b1190	3.48	2.30	Alanine racemase ^b
<i>deoB</i>	b4383	3.25	2.83	Deoxyribouratase, phosphopentomutase ^b
<i>lpxC</i>	b0096	3.73	2.83	UDP-3-O-acyl N-acetylglucosamine deacetylase; lipid A biosynthesis ^b
<i>ptsG</i>	b1101	4.59	3.03	PTS family enzyme IIC, glucose-specific ^b
<i>rplB</i>	b3317	3.73	2.30	50S ribosomal subunit protein L2 ^b
<i>rplD</i>	b3319	3.25	2.14	50S ribosomal subunit protein L4 ^b
<i>rplF</i>	b3305	5.28	3.48	50S ribosomal subunit protein L6 ^b
<i>rplJ</i>	b3985	3.73	3.25	50S ribosomal subunit protein L10 ^b
<i>rplW</i>	b3318	3.73	2.46	50S ribosomal subunit protein L23 ^b
<i>rpmC</i>	b3312	3.03	2.14	50S ribosomal subunit protein L29 ^b
<i>rpsC</i>	b3314	3.73	2.64	30S ribosomal subunit protein S3 ^b

<i>rpsJ</i>	b3321	3.48	2.00	30S ribosomal subunit protein S10 ^b
<i>rpsS</i>	b3316	4.00	2.14	30S ribosomal subunit protein S19 ^b
<i>ytfK</i>	b4217	3.25	2.46	Function Unknown ^b
<i>ibpA</i>	b3687	2.46	2.14	Chaperone, heat-inducible protein of HSP20 family ^a
<i>ibpB</i>	b3686	2.14	Nd	Chaperone, heat-inducible protein of HSP20 family ^a

Nd: not detected in the array. ^a - genes also highly expressed under hydrogen peroxide exposure²; ^b - genes highly expressed under paraquat exposure³; ^c - Gene triggered by *soxRS* response in *E. coli*⁴

Table 2. Gene expression potentially related to membrane damage and repair

Gene	b no.	SWNT Fold Change	MWNT Fold Change	Gene Function
Stress Response				
<i>hslV</i>	b3932	4.59	3.03	Peptidase component of the HslUV protease ^a
<i>ibpA</i>	b3687	2.46	2.14	Small heat shock protein ^a
<i>pphA</i>	b1838	2.64	1.87	Serine/threonine-specific protein phosphatase 1, signals protein misfolding ^a
<i>cspA</i>	b3556	4.59	2.14	Major cold shock protein 7.4, transcription antiterminator of <i>hns</i> , single stranded DNA-binding property ^a
<i>clpB</i>	b2592	3.73	3.25	Heat shock protein ^b
<i>lon</i>	b0439	2.64	2.14	ATP-dependent protease, heat shock protein ^b
<i>rpoH</i>	b3461	3.03	2.64	RNA polymerase, sigma(32) factor; regulation of proteins induced at high temperatures ^b
<i>dnaK</i>	b0014	4.29	3.48	DnaK-homologue chaperone Hsc66 ^b
<i>groEL</i>	b4143	3.73	3.03	Chaperone Hsp60, peptide-dependent ATPase, heat shock protein ^b
<i>recA</i>	b2699	2.30	1.74	DNA strand exchange and renaturation, DNA-dependent ATPase, DNA- and ATP-dependent coprotease ^b
<i>grpE</i>	b2614	2.83	2.46	Heat shock protein; protein repair ^b
<i>rpsF</i>	b4200	2.64	Nd	30S ribosomal subunit protein S6 ^b
<i>groES</i>	b4142	3.73	3.73	GroES, 10 Kd chaperone binds to Hsp60 in pres. Mg-ATP, suppressing its ATPase activity ^b
<i>clpP</i>	b0437	4.00	3.03	ATP-dependent proteolytic subunit of clpA-clpP serine protease, heat shock protein ^b
Thiol-disulfide redox system				
<i>trxA</i>	b3781	2.46	2.14	Thioredoxin 1, redox factor, carrier protein ^a
<i>nrdI</i>	b2674	1.32	1.62	Thioredoxin 2 ^a
<i>nrdH</i>	b2673	2.30	2.14	Stimulates ribonucleotide reduction ^a
Fe-S cluster status				
<i>fnr</i>	b1334	3.48	2.14	Transcriptional regulator of aerobic and anaerobic respiration and osmotic balance (cyclic AMP-binding family) ^a
<i>iscR</i>	b2531	4.00	3.03	Repressor of the <i>iscRSUA</i> operon, involved in assembly of Fe-S clusters ^a
<i>hscA</i>	b2526	2.64	Nd	Chaperone (Hsp70 family), involved in assembly of Fe-S clusters ^a
<i>fdx</i>	b2525	2.46	1.87	[2Fe-2S] ferredoxin, electron carrier protein, involved in assembly of Fe-S ^a
Fatty acid biosynthesis				
<i>fabZ</i>	b0180	2.83	1.87	(3R)-hydroxymyristol acyl carrier protein dehydratase ^c
<i>fabH</i>	b1091	2.64	2.00	3-oxoacyl-(acyl-carrier-protein) synthase III; acetylCoA ACP transacylase ^c
<i>fabI</i>	b1288	2.64	1.74	enoyl-(acyl-carrier-protein) reductase (NADH) ^c
<i>fabD</i>	b1092	2.30	2.14	malonyl-CoA-(acyl-carrier-protein) transacylase ^c
<i>fabA</i>	b0954	2.14	Nd	beta-hydroxydecanoyl thioester dehydrase, trans-2-decenoyl-ACP isomerase ^c
<i>fabB</i>	b2323	2.00	1.52	3-oxoacyl-(acyl-carrier-protein) synthase I ^c
<i>fabF</i>	b1095	2.00	1.52	3-oxoacyl-(acyl-carrier-protein) synthase II ^c

<i>fabG</i>	b1093	3.73	2.00	3-oxoacyl-(acyl-carrier-protein) reductase ^c
Beta-oxidation pathway				
<i>fadA</i>	b3845	2.14	Nd	thiolase I; 3-ketoacyl-CoA thiolase; acetyl-CoA transferase ^d
<i>fadD</i>	b1805	5.28	1.87	acyl-CoA synthetase ^d
<i>fadL</i>	b2344	3.48	2.00	long-chain fatty acid transport protein (outer membrane flp protein) ^d
<i>fadB</i>	b3846	3.25	2.46	4-enzyme protein: 3-hydroxyacyl-CoA dehydrogenase; 3-hydroxybutyryl-CoA epimerase; delta(3)-cis-delta(2)-trans-enoyl-CoA isomerase; enoyl-CoA hydratase ^d
<i>aidB</i>	b4187	3.03	1.52	putative acyl coenzyme A dehydrogenase ^d
Miscellaneous genes related to membrane integrity				
<i>rbsD</i>	b3748	4.00	Nd	membrane-associated component of high-affinity D-ribose transport system, membrane ^a
<i>rpoE</i>	b2573	3.73	3.25	Sigma E factor of RNA polymerase, response to periplasmic stress ^a
<i>hns</i>	b1237	4.59	3.25	Transcriptional regulator, DNA-binding protein HLP-II increases DNA thermal stability ^a
<i>otsB</i>	b1897	3.48	3.25	Trehalose-6-phosphate phosphatase, osmoregulation ^a
<i>yebF</i>	b1847	2.83	2.00	Conserved protein ^a
<i>yafQ</i>	b0225	2.46	1.87	Conserved protein ^a
<i>rpsU</i>	b3065	2.30	1.74	30S ribosomal subunit protein S21 ^a
<i>rhoL</i>	b3782	3.73	1.40	rho operon leader peptide ^a
<i>pal</i>	b0741	3.25	2.46	peptidoglycan-associated lipoprotein ^e
<i>tolA</i>	b0739	1.74	1.52	acetolactate synthase I, valine sensitive, small subunit ^e
<i>tolB</i>	b0740	2.83	2.30	periplasmic protein involved in the tonB-independent uptake of group A colicins ^e
<i>ybgF</i>	b0742	3.03	Nd	Function unknown ^e
<i>phoP</i>	b1130	3.48	1.87	transcriptional regulatory protein ^e
<i>phoQ</i>	b1129	3.48	1.87	sensor protein PhoQ ^e

Nd =not detected; ^a – genes also highly expressed under high pressure damages⁵; ^b – genes also expressed in proteomics work under higher pressure damages⁶; ^c – genes involved in fatty acid biosynthesis⁷; ^d – involved in fatty acid uptake and catabolism⁸; ^e – genes known to be involved in maintaining the integrity of the outer membrane^{10,11}.

References

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