Supporting Information

The SI contains 13 pages of additional information, including 4 texts, 3 figures, and 5 tables.

TEXTS

Text S1. Medium, chemicals and reagents

Luria-Bertani (LB) liquid medium (tryptone, 10g/L; yeast extract, 5g/L; NaCl, 10 g/L; PH 7.2-7.4) and LB solid medium (15g/L of agar) were used in this study. The chemicals used in this study were all reagent-grade. The reverse transcription kits (Directory No. 2680A), RNAisoPlus kits (Directory No. D9108A), SYBR Green I (Directory No. DRR420A), and 2×SYBR Premix Ex Taq were purchased from TaKaRa (TaKaRa, Dalian, China). Kanamycin (Km), chloramphenicol (Chl), C_{60} and thiourea (CH_4N_2S, TU) were provided by TCI (TCI, Tokyo, Japan) and AGAR was purchased from Fisher Scientific. The 2', 7'-dichlorofluorescein diacetate (DCFH-DA) was purchased from Invitrogen (Invitrogen, Shanghai, China). Tryptone, yeast extract, sodium chloride (NaCl), potassium dihydrogen phosphate (KH_2PO_4), sodium dihydrogen phosphate (Na_2HPO_4), potassium chloride (KCl), sodium hydroxide (NaOH), and concentrated hydrochloric acid were purchased from Sigma-Aldrich (Sigma-Aldrich, Shanghai, China).

Text S2. Preparation and characterization of nC_{60}

First, 4 mg C_{60} solid powder was fully dissolved into 18 mL toluene. Then 144 mL ultra-pure water was added, and continuously ultrasonicated for 24 h (40 kHz, 180 W) in a sealed manner. Then it was ultrasonicated for another 24 h under the condition of 40 °C water bath in an exposed manner, in order to let the toluene volatilize from the solution. To ensure no toluene remained in the solution, after the sonication, we kept the stock solution in a water bath with a temperature of 60 ° C and replenished it with ultra-pure water repeatedly. Then the stock solution was filtered through a 0.45 μm teflon filter, and the concentrations of nC_{60} solution was measured by ultraviolet absorption at 334 nm (Yin et al., 2016). The centrifuge tube was shaken at a speed of 200 rpm for 30 min. Because of the very low melting point of toluene, - 94.9 °C, we put the solution into a -80 °C fridge for 30 minutes to make the water layer frozen and the rest toluene poured out. After the ice melted, 1.5 mL toluene was added for extraction; the above operation was repeated for a total of 3 times. The extract was mixed and the absorbance value at 334nm was determined. The concentration of C_{60} mother solution was obtained by combining with the standard curve of C_{60}. The nC_{60} mother solution was diluted and ultrasonicated for 30 min, and the particle size distribution was determined by using Malvern Instruments' nanoparticle size - Zeta potentiometric analyzer.

Text S3. Effect of nC_{60} on transformation of plasmid-carried AR Gs

E. coli s17-1 was cultured overnight in LB liquid medium (containing 20 mg/L Chl) at 37 °C with 180 rpm shaking.

The plasmids were isolated and purified using Plasmid Minispin HP Kit Ver.2 (Vigorous Biotechnology Beijing Co.,Ltd, Beijing). *E. coli* K12 MG1655 grown overnight in LB liquid medium (containing 100 mg/L Km) for 10 h at 37 °C were pelleted at 8000 rpm for 5 min, then re-suspended with LB liquid medium. The bacterial cultures and nC_{60} mother liquor were mixed in a sterile 1.5 mL microtube and briefly vortexed. Then the microtube was incubated in an airtight dark incubator at 37 °C for specified time periods. The bacterial cultures were centrifuged at 8000 rpm for 3 min, followed by washing three times with ultra-pure water. Then, the bacteria resuspended in 98 µL of LB liquid medium. Two microliters of different concentrations of the plasmid were added to 1.5 mL microtubes containing 98 µL bacteria treated with nC_{60} as described above. Meanwhile, we set up a control group. In the control group, two microliters of sterile purified water instead of the plasmid were added to microtubes containing 98 µL bacterial cells treated with nC_{60}. The mixture was vortexed for 2 min. Subsequently, 900 µL of LB was added to each microtube, which was placed in a thermostat shaker maintained at 37 °C with shaking at 180 rpm for 1 h in an airtight dark incubator to allow bacteria to recover. The bacterial solution was properly diluted in PBS and coated on LB solid plates containing different antibiotics to determine the numbers of zygotes and recipient strains. Finally, both the ratio of nC_{60} to transformation rate of ARGs (the ratio of the number of zygote colonies to the number of recipient strain colonies) and the transformation fold relative to the control group were calculated. (Ding et al., 2016).

In addition, we also verified whether the plasmid can be transferred into the receptive cells. After treatment with $CaCl_2$ (100 mmol/L), the receptor cells were treated with heat shock (42 °C heat shock for 90 seconds). After heat shock, the receptor cells need to grow in LB liquid medium without antibiotics for at least half an hour. The bacterial solution was properly diluted in PBS and coated on LB solid plates containing different antibiotics to determine the numbers of zygotes and recipient strains.

Text S4. RNA extraction and quantification

RNA was extracted by the RNAiso Plus kits. 1 mL conjugate mixture exposed to nC_{60} was mixed with 1 mL RNAiso Plus and centrifuged at 12,000 x *g* for 5 min at 4 °C. The supernatant was transferred to a new 1.5 mL tube which 0.2 mL chloroform was added. The tube was centrifuged at 12,000 x *g* for 15 min at 4 °C. And then, the supernatant was transferred to a new centrifuge tube which 0.5 mL isopropanol was added. After that, the centrifuge tube was centrifuged at 12,000 x *g* for 10 min at 4 °C. The precipitate was washed with 1 mL 75% alcohol and centrifuged at 7500 x *g* for 5 min at 4 °C. After drying, the remained precipitate was dissolved in appropriate amount of RNase-free aqueous solution. The concentration was measured with spectrophotometer.

FIGURES

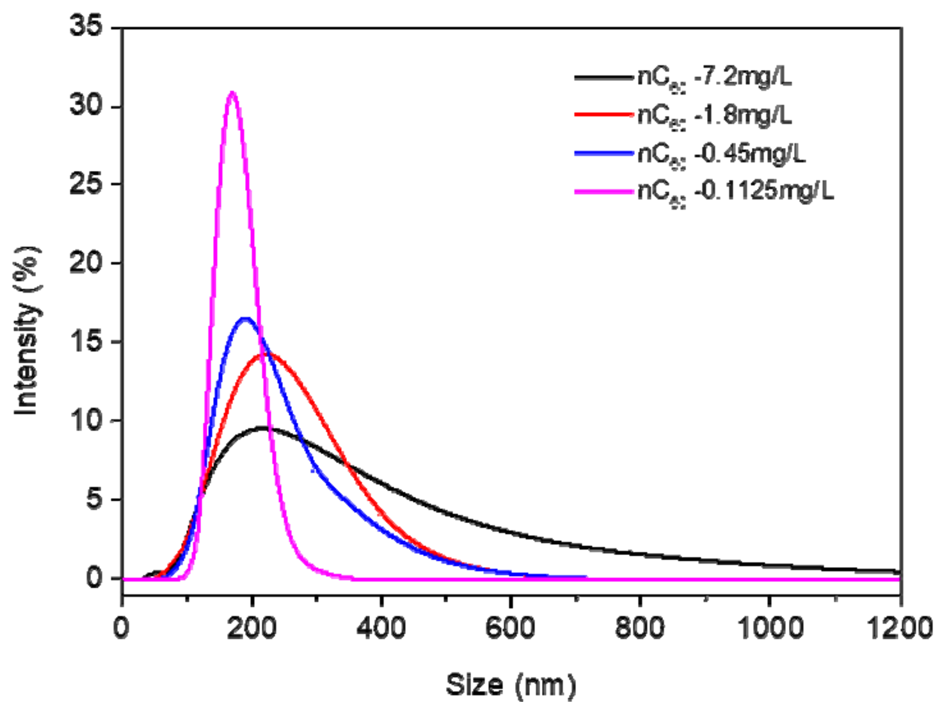

Fig. S1 Intensity-averaged particle size distributions of different concentrations of nC_{60} aqueous suspensions in PBS.

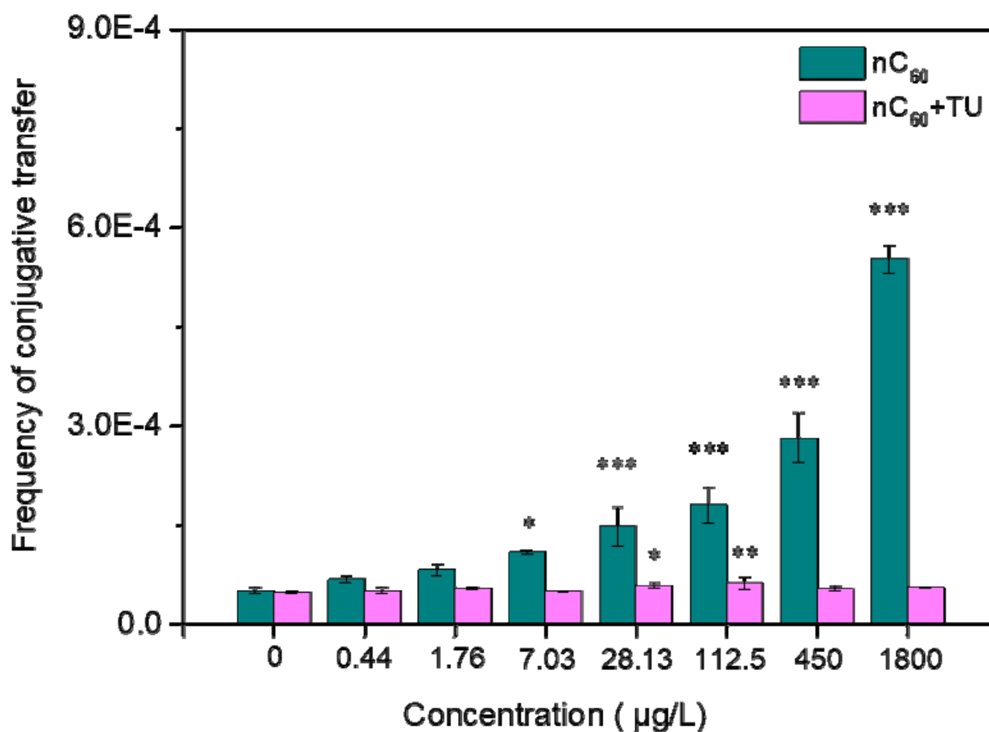

Fig. S2 Frequency in conjugation between two *E. coli* strains upon exposure to nC_{60}. nC_{60} had significant effects on the conjugative transfer of ARGs between *E. coli* strains (ANOVA, $P < 0.05$); significant differences between individual nC_{60} or nC_{60}+TU treated groups and the control (0 μg/L of nC_{60}) were tested with ANOVA (LSD); * ($P < 0.05$), ** ($P < 0.01$) and *** ($P < 0.001$).

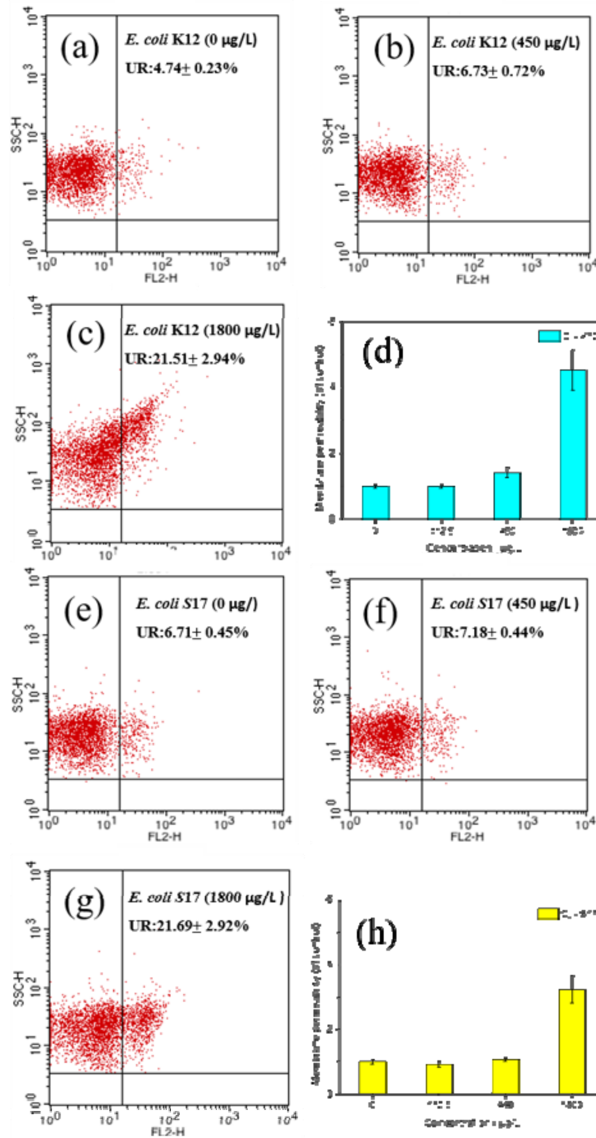

Fig. S3 Flow cytometric analysis of *E. coli* K12 after incubation in control medium (a), 450 μg/L nC_{60} (b), and 1800 μg/L nC_{60} (c). Flow cytometric analysis of *E. coli* S17 after incubation in control medium (e), 450 μg/L nC_{60} (f), and 1800 μg/L nC_{60} (g). Upper-right quadrant: PI-positive as the permeability-increasing cells. Upper-left quadrant: negative-signal as normal cells. (d) and (f) describe the fold of the experimental groups relative to the control group for *E. coli* K12 and *E. coli* S17.

TABLES

Table S1. Inhibition rates of nC_{60} on *E. coli* K12 MG1655 and *E. coli* S17-1

Concentrations (μg/L)	*E. coli* K12	*E. coli* S17-1
3600	0.47±0.05	0.44±0.07
1800	0.27±0.06	0.29±0.03
900	0.20±0.03	0.23±0.02
450	0.18±0.03	0.18±0.05
225	0.02±0.08	0.11±0.04
112.5	0.00±0.06	0.06±0.02

Table S2. The exposure sub-inhibitory concentration of nC_{60}

Number	1	2	3	4	5	6	7	8
Concentration (μg/L)	0	0.44	1.76	7.03	28.13	112.5	450	1800

Table S3. The number of zygotes and recipient strains at different concentration of nC_{60}

concentration of nC_{60} (μg/L)	Mean (Number of zygotes)	SD (Number of zygotes)	Mean (Number of recipient strains)	SD (Number of recipient strains)
0	1.17E+04	1.07E+03	2.30E+08	1.28E+07
0.44	1.11E+04	9.11E+02	1.64E+08	3.15E+07
1.76	1.06E+04	1.27E+03	1.30E+08	2.02E+07
7.03	2.03E+04	4.86E+02	1.87E+08	6.16E+07
28.13	2.00E+04	3.97E+03	1.36E+08	2.79E+07
112.5	3.12E+04	4.82E+03	1.73E+08	1.60E+07
450	4.60E+04	6.16E+03	1.63E+08	3.09E+07
1800	1.01E+05	4.02E+03	1.84E+08	5.48E+07

Table S4. The number of zygotes of transformation under different conditions

Number of *E.coli* K12	concentration of nC_{60} (μg/L)	Quality of plasmid（ng）	Time（h）	Mean (Number of zygotes)	SD (Number of zygotes)
1×10^8	0	100	9	0	0
1×10^8	1800	25	9	0	0
1×10^8	1800	50	9	0	0
1×10^8	1800	100	9	0.33	0.47
1×10^9	0	100	9	0	0
1×10^9	1800	100	9	0.33	0.47
1×10^9 (Ca^{2+})	0	30	0.5	90	7.07
1×10^9 (Ca^{2+})	0	100	0.5	1973.33	131.59

Table S5. The primer sequences in the study

Category	Gene	Primer	Sequence of Primer (5'-3')	Length
Global regulator genes of HGT	***korA***	*korA-F*	TCGGGCAAGTTCTTGTCC	147 bp
		korA-R	GCAGCAGACCATCGAGATA	
	korB	*korB-F*	CTGGTCGGCTTCGTTGTA	149 bp
		korB-R	TGAAGTCACCCATTTCGGT	
	trbA	*trbA-F*	TGGAAACTCCCCTACCTCTT	120 bp
		trbA-R	CCACACTGATGCGTTCGTAT	
Mating pair formation (Mpf) system genes	***trbBp***	*trbBp-F*	CGCGGTCGCCATCTTCACG	131 bp
		trbBp-R	TGCCCGAGCCAGTACCGCCAATG	
	traF	*traF-F*	GGCAACCTCGTCGCCTTTA	118 bp
		traF-R	GCAAGTCGGCGTGTTTTCG	
DNA transfer and replication (Dtr) system genes	***trfAp***	*trfAp-F*	GAAGCCCATCGCCGTCGCCTGTAG	183 bp
		trfAp-R	GCCGACGATGACGAACTGGTGTGG	
	traJ	*traJ-F*	GCCCGTGATTTTGTAGCCC	151 bp
		traJ-R	TGAAACCAAGCCAACCAGGAA	
Outer membrane protein genes	***ompA***	*ompA-F*	TGAGCCTGGGTGTTTCCTA	167 bp
		ompA-R	CAGAGCAGCCTGACCTTCC	
	ompF	*ompF-F*	GGTCTGCGTCCGTCCAT	99 bp
		ompF-R	GGTTGCGCCCACTTCA	
	ompC	*ompC-F*	AAGTAGTAGGTAGCACCAACATCA	163 bp
		ompC-R	GGGCGAACAAAGCACAGAA	
Category	Gene	Primer	Sequence of Primer (5'-3')	Length
Oxidative stress-related genes	***rpoS***	*rpoS-F*	TTTTACCACCAGACGCAAGT	184 bp
		rpoS-R	GGAACTGTTATCGCAGGGAG	
	soxS	*soxS-F*	AATCGGACGCTCGGTGGT	132 bp
		soxS-R	AAATCAGGCTATTCAAAGTGGT	
	soxR	*soxR-F*	ATTGGTGAAGCGTTTGGC	145 bp
		soxR-R	CAATACATCCGTCCAGTTCGT	
	oxyR	*oxyR-F*	CGTGCTGCGTGAGGTGA	188 bp

		oxyR-R	GTAACTGGTGGGTCTGTGCTT	
SOS response-related genes	***recA***	*recA-F*	CGCTTTCGGCGTCAGT	125 bp
		recA-R	ACAACCTGCTGTGCTCCC	
	lexA	*lexA-F*	AATGCTGATTTCCTGCTGC	175 bp
		lexA-R	CGACTTTATTGCCCTGTTTT	
	umuD	*umuD-F*	TGACGGCGAGTTTACGG	117 bp
		umuD-R	CCAAAGACATCCAGCGTAT	
16S rRNA	***16S rRNA***	*16s-F*	CCTACGGGAGGCAGCAG	194 bp
		16s-R	ATTACCGCGGCTGCTGG	

References

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