

Electronic Supplementary Information

Engineering the surface of graphene oxide with bovine serum albumin for improved biocompatibility in *Caenorhabditiselegans*

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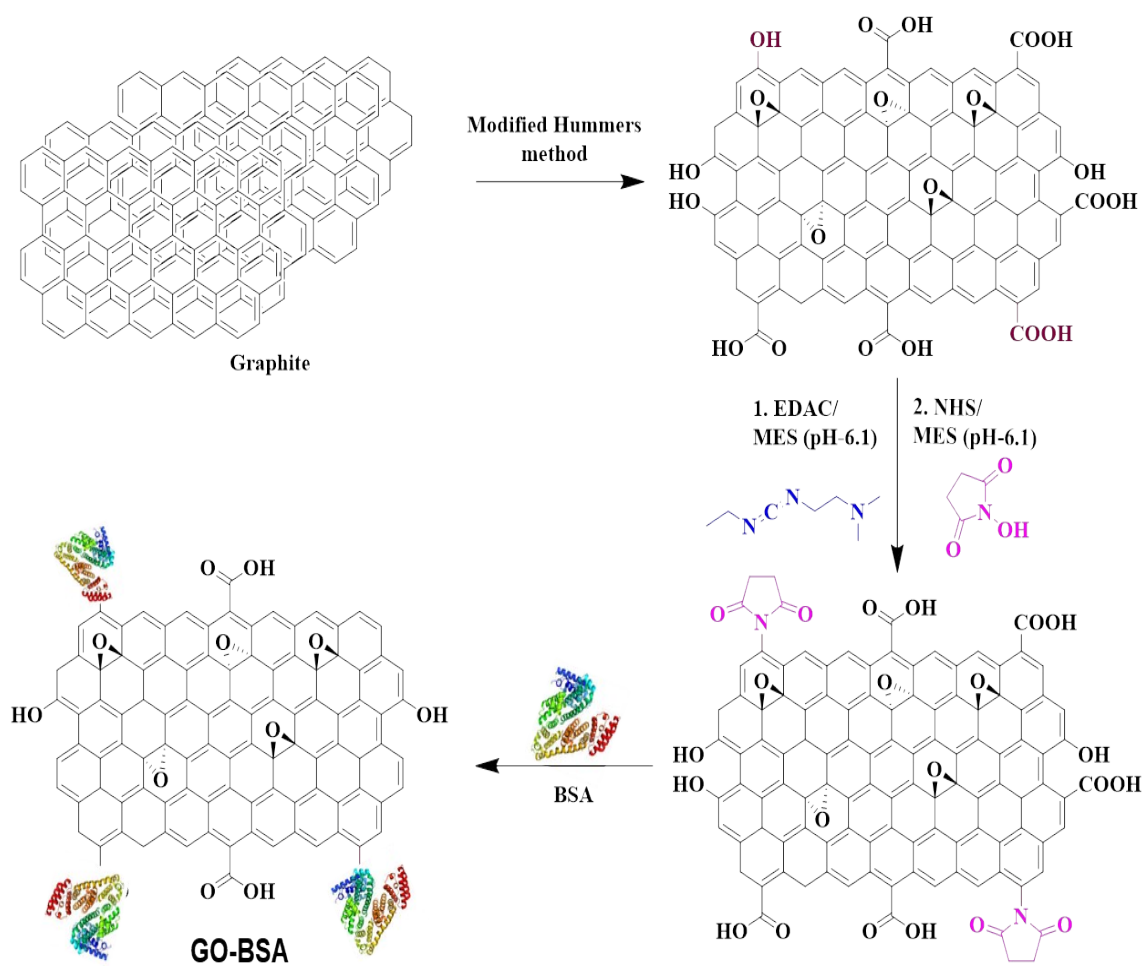


Figure S1. Schematic representation for the preparation of bovine serum albumin decorated graphene oxide (GO-BSA).

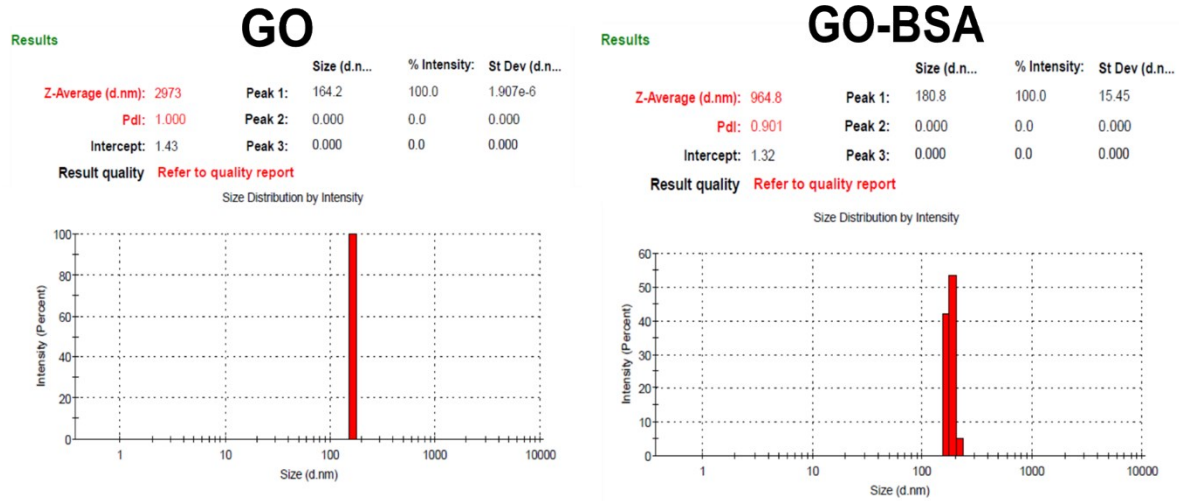


Figure S2. Size distribution analysis of GO and GO-BSA.

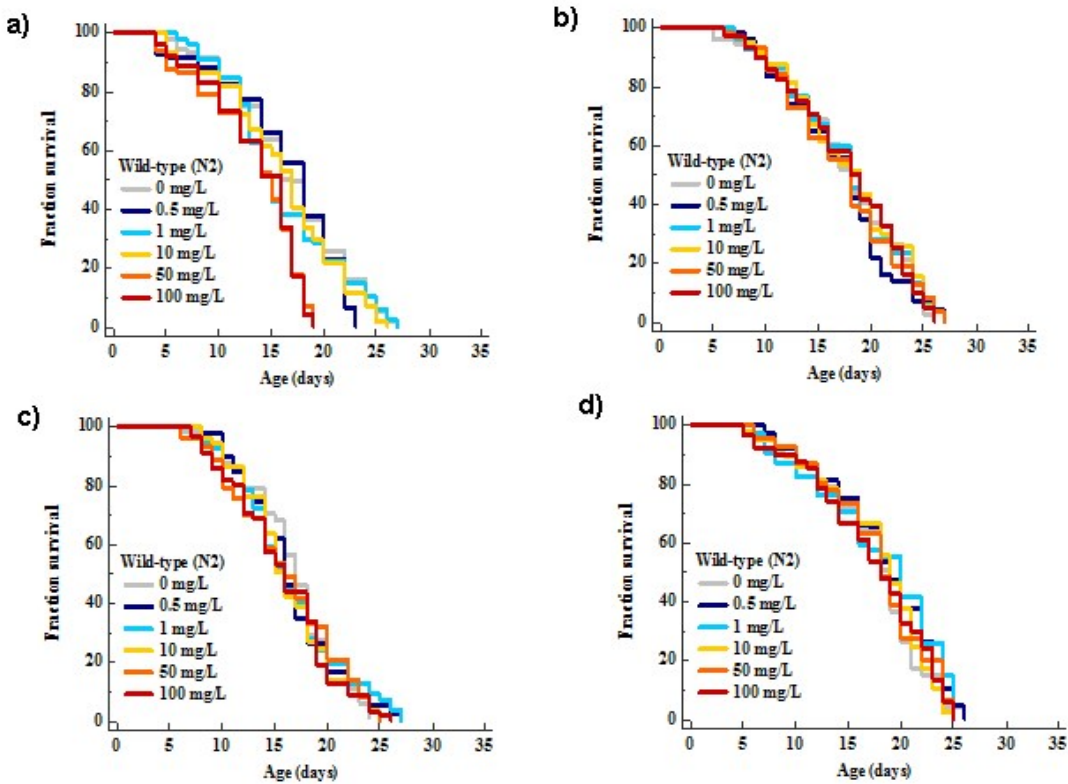


Figure S3. Effect of GO and GO-BSA exposure on the lifespan of wild-type *C. elegans*. Survival curves of wild-type worms under (a) acute GO (b) acute GO-BSA, (c) Prolonged GO, and (d) prolonged GO-BSA exposures at 20°C. The representative Kaplan–Meier survival curves from three independent biological trials were presented.

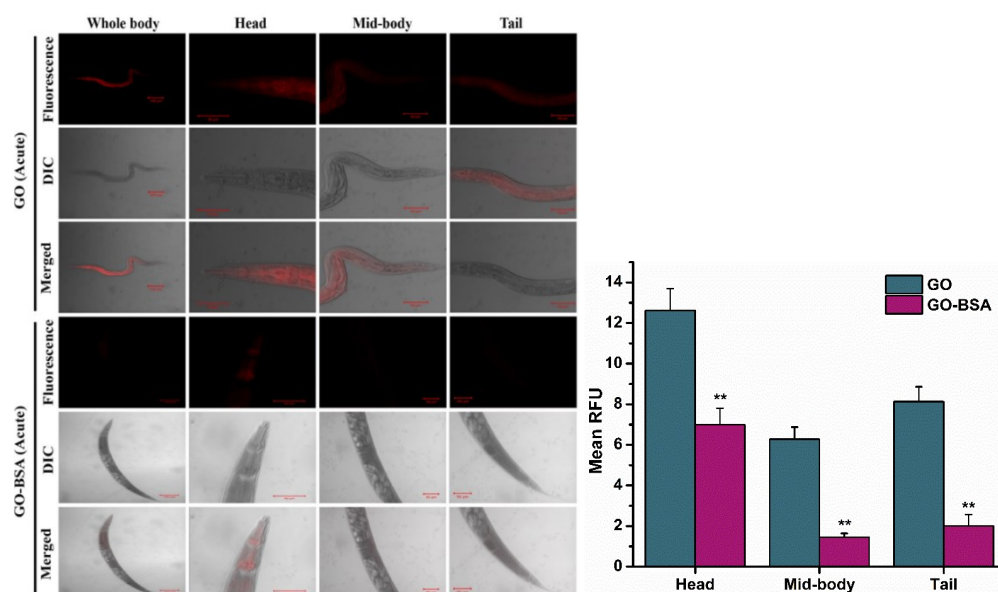


Figure S4. Distribution of GO and GO-BSA after acute exposure in *C. elegans*. Fluorescence micrograph showing the bio-distribution of Rhodamine-B loaded GO and GO-BSA after acute exposure of 100 mg L⁻¹ in nematodes and the quantification of Rhodamine-B loaded GO and GO-BSA fluorescence in different parts of nematodes. Error bar indicates the mean value of three independent experimental data (n≥20/experiment). **p<0.01.

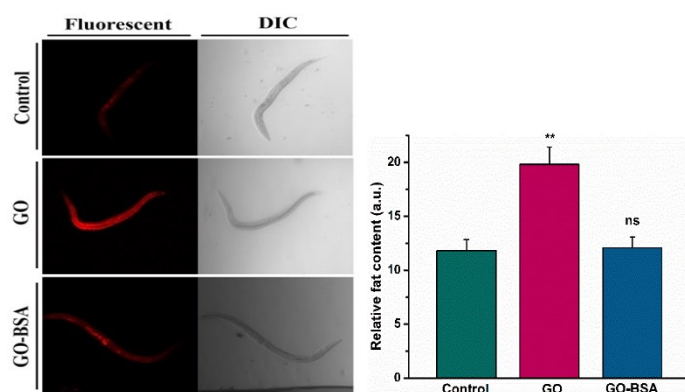


Figure S5. Effect of GO and GO-BSA (100 mg L⁻¹) on intestinal permeability in *C. elegans*. Fluorescent micrograph showing the intestinal permeability of GO and GO-BSA after acute exposure and its quantification of relative fluorescent intensity. Error bar indicates the mean value of three independent experimental data (n≥30/experiment). **p<0.01, ns-not significant.

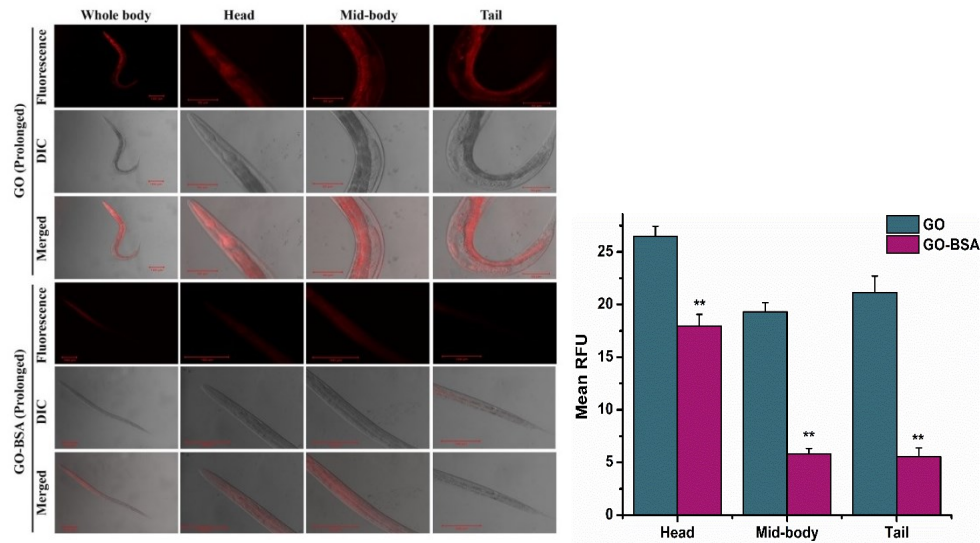


Figure S6. Distribution of GO and GO-BSA (100 mg L^{-1}) in wild-type *C. elegans*. Fluorescence micrograph showing the bio-distribution of Rhodamine-B loaded GO and GO-BSA after prolonged exposure in wild-type nematodes and its representative quantification of Rhodamine-B loaded GO and GO-BSA fluorescence in different parts of nematodes. Error bar indicates the mean value of three independent experimental data ($n \geq 20/\text{experiment}$). ** $p < 0.01$.

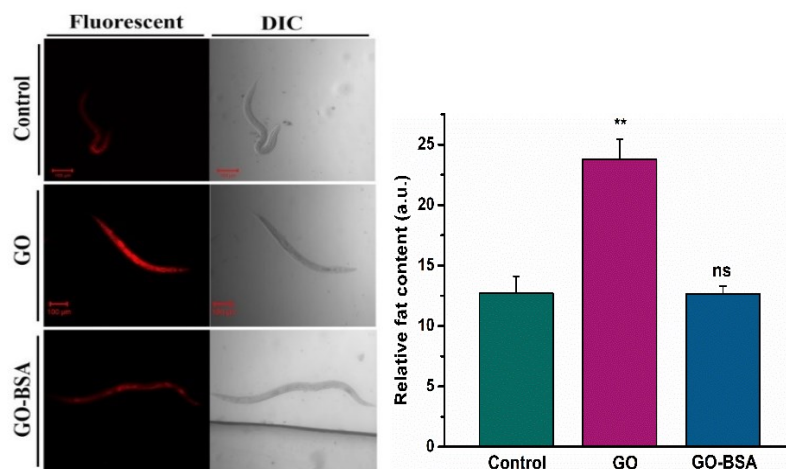


Figure S7. Effect of GO and GO-BSA (100 mg L^{-1}) on intestinal permeability in *C. elegans* (N2 worms). (a) Fluorescence micrograph showing the intestinal permeability of GO and GO-BSA after prolonged exposure in *C. elegans* and its representative quantification of relative fluorescent intensity of Nile red in exposed nematodes. Error bar indicates the mean value of three independent experimental data ($n \geq 20/\text{experiment}$). ** $p < 0.01$, ns-not significant.

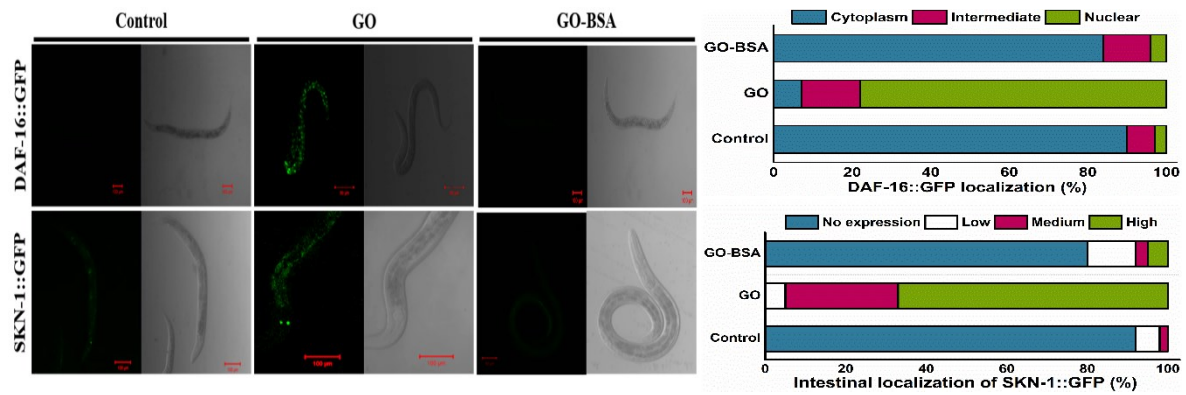


Figure S8. Effect of GO and GO-BSA (100 mg L⁻¹) on expression of antioxidant defense genes in *C. elegans*. Representative fluorescent image showing the localization of *daf-16::GFP* and *skn-1::GFP* in *C. elegans* and its respective quantification of nuclear localization after prolonged exposure to GO and GO-BSA. Error bar indicates the mean value of three independent experimental data (n=20/experiment). **p<0.01.

Table S1. Survival rate of wild-type *C. elegans* cultured on NGM plates carrying either GO or GO-BSA. The mean lifespan of *C. elegans* were calculated using Kaplan–Meir survival analysis and significance levels were estimated by long-rank test in Medcalc statistical tool.

Treatment		Mean survival (Mean±SEM)	Maximum lifespan (days)	Sample size (N)	Censored	% Change	p value
GO (acute)	Concentration						
	0 mg/L	16.723±0.387	24	136	16		
	0.5 mg/L	16.456±0.405	27	130	9	(-) 1.60	0.6067
	1 mg/L	16.475±0.432	27	142	15	(-) 1.48	0.9475
	10 mg/L	16.136±0.399	25	126	7	(-) 3.51	0.3777
	50 mg/L	16.028±0.466	26	130	13	(-) 4.16	0.7338
	100 mg/L	15.750±0.401	26	148	10	(-) 5.82	0.3646
GO (prolonged)	0 mg/L	16.908±0.476	27	145	17		
	0.5 mg/L	16.348±0.496	23	128	17	(-) 3.31	0.1391
	1 mg/L	16.074±0.486	27	132	16	(-) 4.93	0.2013
	10 mg/L	15.996±0.493	26	141	13	(-) 5.39	0.1393
	50 mg/L	13.382±0.375	19	154	15	(-) 20.85	0.0001
	100 mg/L	13.630±0.390	19	125	14	(-) 22.39	0.0001
GO-BSA (acute)	0 mg/L	17.566±0.514	26	126	16		
	0.5 mg/L	17.036±0.480	27	122	13	(-) 3.02	0.1156
	1 mg/L	17.709±0.478	26	135	17	(+) 0.81	0.3697
	10 mg/L	17.807±0.499	27	130	18	(+) 1.37	0.5234
	50 mg/L	17.277±0.524	26	118	13	(-) 1.65	0.3149
	100 mg/L	17.730±0.498	27	132	19	(+) 0.93	0.2757
GO-BSA (prolonged)	0 mg/L	17.547±0.453	26	127	16		
	0.5 mg/L	18.376±0.460	26	138	17	(+) 4.72	0.1228
	1 mg/L	17.973±0.570	25	118	22	(+) 2.43	0.0934
	10 mg/L	17.724±0.461	25	133	17	(+) 1.01	0.7234
	50 mg/L	17.723±0.447	25	138	19	(+) 1.00	0.7458
	100 mg/L	17.314±0.474	25	145	20	(-) 1.33	0.9751

Table S2. Primers used for qRT-PCR analysis.

S. No	Gene	Sequence (5'→3')	
		Forward	Reverse
1	<i>act-1</i>	ACCATGTACCCAGGAATTGC	TGGAAGGTGGAGAGGGAAG
2	<i>ced-3</i>	ACGGGAGATCGTGAAAGC	AGAGTTGGCGGATGAAGG
3	<i>ced-4</i>	CTGATGCTAGCCCGTGTGT	CGTTGCTGGATTTCCACTGC
4	<i>ced-9</i>	GCTGTTTCATCAAAACGCGGA	ATCGACCACCGTCTGTTCTG
5	<i>cep-1</i>	GTCGGAACCCAAGTGTAT	CGAGTTACCTGCCTACAA
6	<i>egl-1</i>	CACCTTTGCCTCAACCTC	TTGGAGCCGATCTCGTAG
7	<i>hus-1</i>	GCGGCAATCGACGTGTTTAT	CCGGGCAGAACACGTACTAA