Supporting Information for

Effects of surface-functionalized TiO_2 nanoparticles on copper ion toxicity under simultaneous and sequential exposure conditions

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Zebrafish cultivation and embryo hatching

The breeding temperature was maintained at 28°C, and the lights in the room were programmed to turn on at 8:00 AM and go off at 22:00 PM for a 14-h light cycle. Sodium chloride and sodium bicarbonate were used to adjust the circulating water quality for a conductivity of 500–550 S cm^{-1}, pH of 7.2–7.4, and dissolved organic matter of <1 mg L^{-1}. The zebrafish were fed once a day with freshly hatched brine shrimp, and the amount of each feeding ensured that the zebrafish could finish eating within 5 min.

The male and female fish necessary for mating were taken from the circulating water system and placed in spawning tanks the evening before the zebrafish embryos were to hatch. Each tank included two males and one female. When the lights are turned off, the fish house gets dark, and the zebrafish enters into a period of relative inactivity. The next morning, when the light was turned on, the zebrafish entered the excited state. The fish will generally spawn during the first 2 h after the lights come on. To harvest the embryos, remove the insert from the spawning tank. Once the embryos and feces have settled to the bottom of the outer tank, slowly decant the water from the spawning tank into the sink. The embryos that are not developing normally or do not appear to be developing were then removed with a wide mouth pipette. For the follow-up experiments, only healthy embryos were used.

Table S1. Properties of three commercial TiO_2 NPs.

Commercial name	VK-T02H	VK-T02SG	VK-T02SY
Nomenclature	H	SG	SY
Crystalline phase	rutile	rutile	rutile
TiO_2 (%)	88-95	82-88	82-88
Coating	SiO_2	SiO_2, silicone oil	SiO_2, stearic acid
Primary crystallite size(nm)	30	30	30
Surface property	hydrophilic	hydrophobic	hydrophobic
Specific surface area ($m^2\ g^{-1}$)	30 ± 15	30 ± 15	30 ± 15

Table S2 Two-way ANOVA testing for the influence of type of nano TiO_2, Cu^{2+} concentration, exposure time and exposure scenarios on the mortality of zebrafish embryo.

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	7.634[a]	87	0.088	31.741	0.000
Intercept	11.474	1	11.474	4150.82	0.000
Type of nano TiO_2	0.027	2	0.013	4.87	0.009
Cu^{2+} conc.	0.853	1	0.853	308.512	0.000
Exposure time	2	3	0.667	241.133	0.000
Exposure scenarios	1.489	2	0.745	269.379	0.000
Type of nano TiO_2 Exposure scenarios	0.057	4	0.014	5.167	0.001
Error	0.619	224	0.002		
Total	20.033	312			
Corrected Total	8.253	311			60.6

a. R Squared = 0.925 (Adjusted R Squared = 0.896)

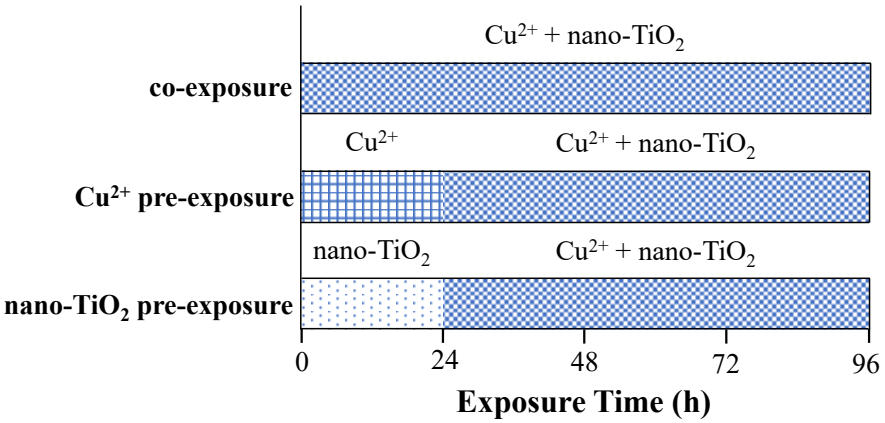

Figure S1 Operative conditions in different exposure scenarios. The concentrations of nano-TiO_2 and Cu^{2+} in individual scenarios were respectively equal to 1 mg-Ti/L and 50 μg-Cu/L.

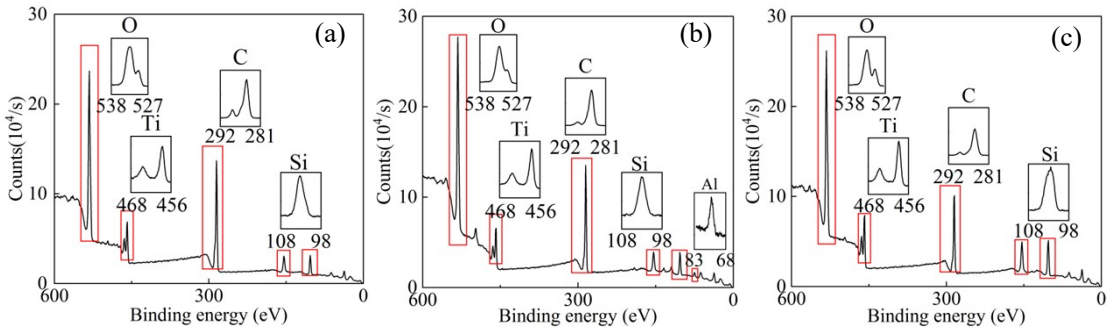

Figure S2 XPS results for three nano-TiO_2 materials: (a) H; (b) SY; (c) SG (Each peak's expanded image is shown in the small image).

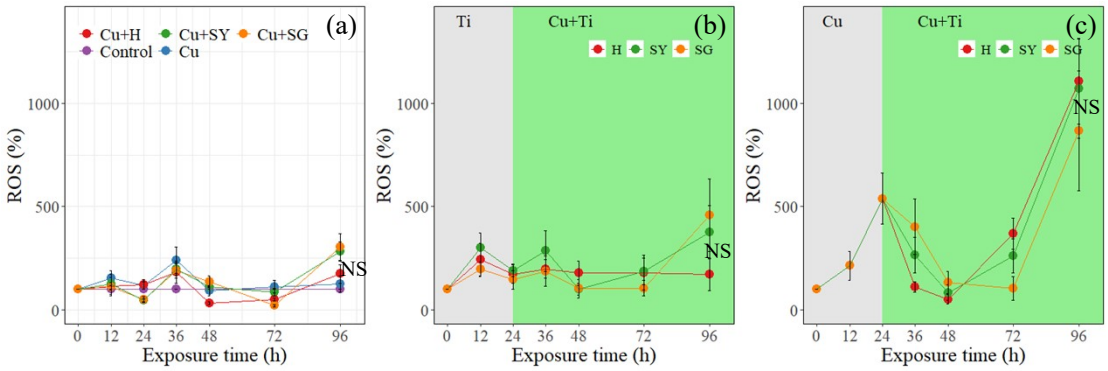

Figure S3 Endogenous ROS content in zebrafish embryos during a 96-h exposure to three types of nano-TiO_2 under different exposure scenarios: co-exposure (a) TiO_2 pre-exposure (b) and Cu^{2+} pre-exposure. Each point is expressed as mean ± standard deviation (n = 3). NS, not significant.

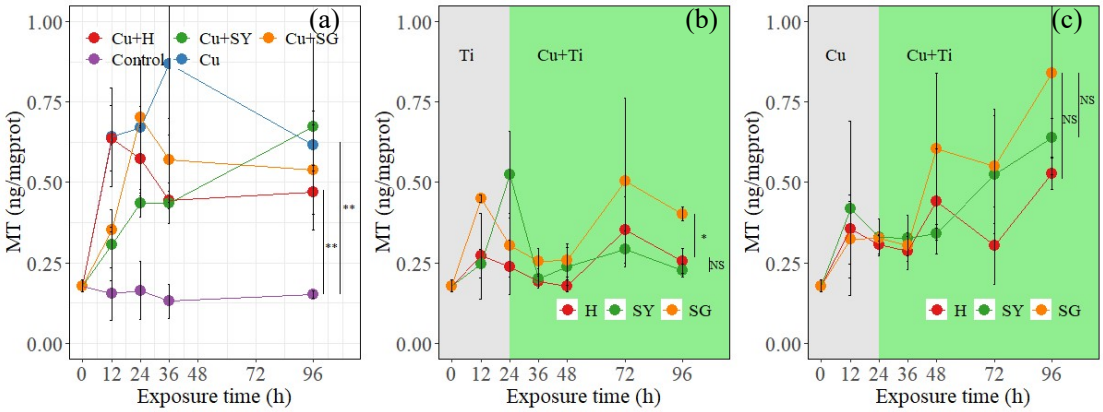

Figure S4 MT activities in zebrafish embryos during a 96-h exposure to three types of nano-TiO_2 under different exposure scenarios: co-exposure (a) TiO_2 pre-exposure (b) and Cu^{2+} pre-exposure. Each point is expressed as mean ± standard deviation ($n = 3$). NS, not significant, *p<0.05; **p<0.01; ***p<0.001 by two-way ANOVA with Tukey's HSD test.

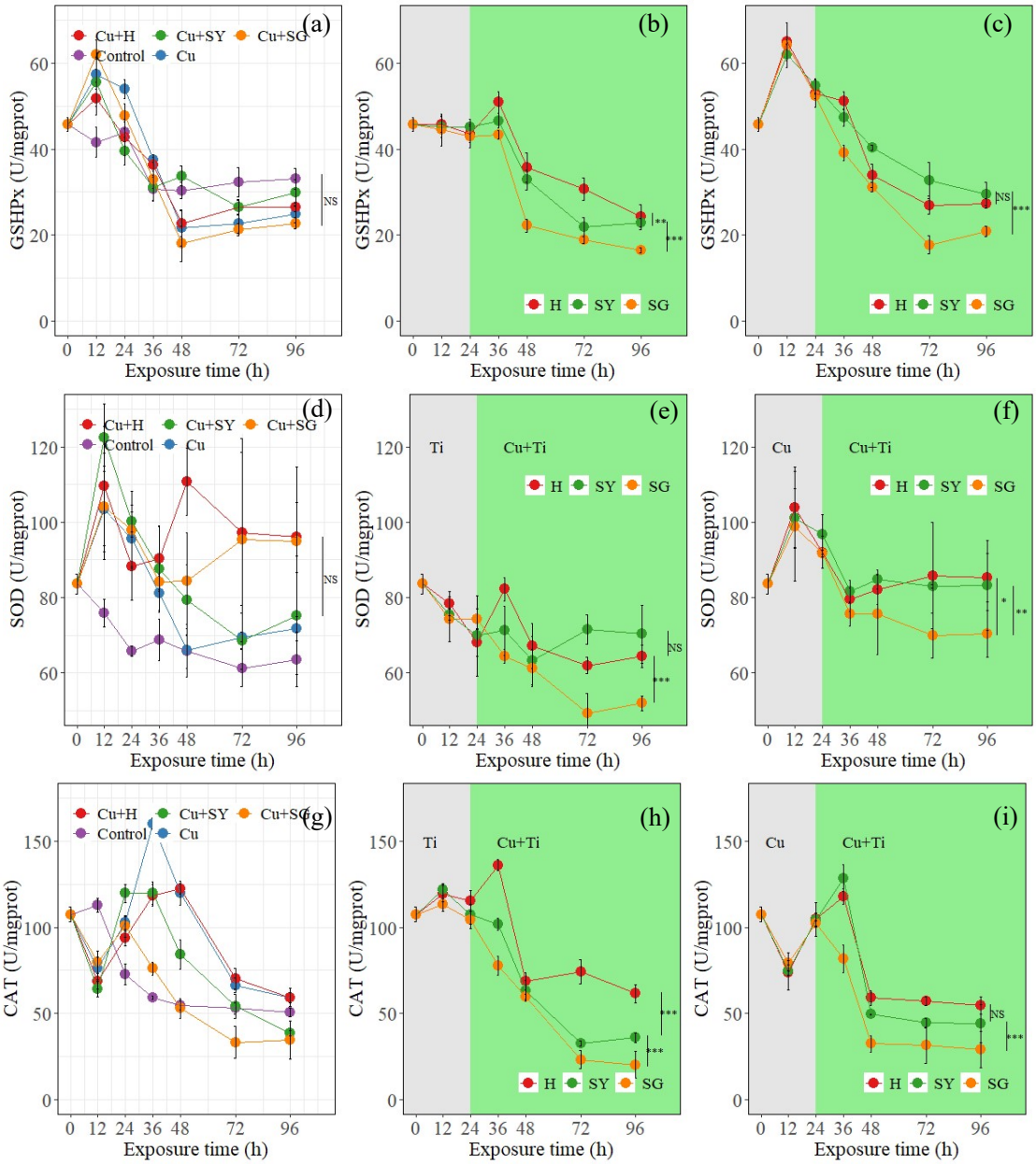

Figure S5 Antioxidant enzyme activities (GSH-Px: (a-c); SOD: (d-f); CAT:(g-i)) in zebrafish embryos during a 96-h exposure to three types of nano-TiO_2 under different exposure scenarios: co-exposure (a, d, g) TiO_2 pre-exposure (b, e, h) and Cu^{2+} pre-exposure (c, f, i). Each point is expressed as mean ± standard deviation (n = 3). NS, not significant, *p<0.05; **p<0.01; ***p<0.001 by two-way ANOVA with Tukey's HSD test.

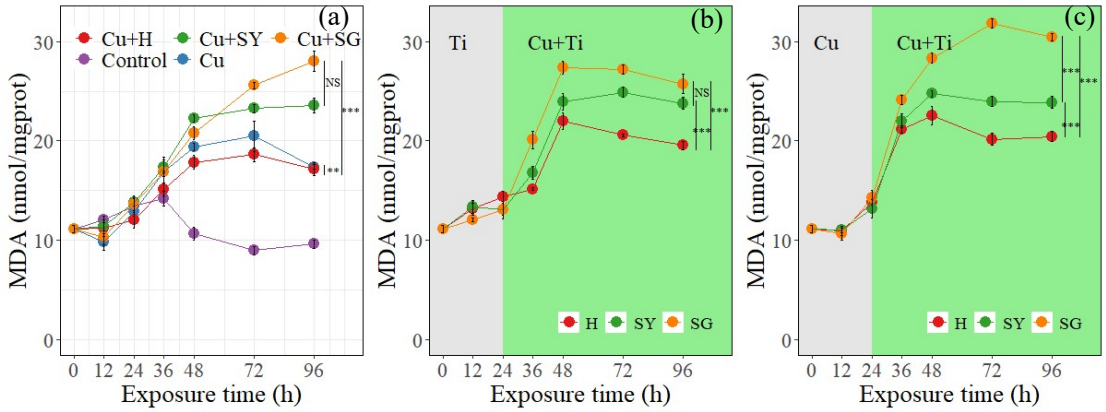

Figure S6 MDA levels in zebrafish embryos during a 96-h exposure to three types of nano-TiO_2 under different exposure scenarios: co-exposure (a) TiO_2 pre-exposure (b) and Cu^{2+} pre-exposure. Each point is expressed as mean ± standard deviation ($n = 3$). NS, not significant, *p<0.05; **p<0.01; ***p<0.001 by two-way ANOVA with Tukey's HSD test.