Electronic Supplementary Material (ESI) for Environmental Science: Nano.

Unique interplay between Zn^{2+} and nZnO determined the dynamic cellular stress in zebrafish cells

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Method to chelate the extracellularly released Zn^{2+}

To capture the extracellularly released Zn^{2+}, chelating effect of EDTA-2Na and cysteine on Zn^{2+} were compared. Influence of EDTA-2Na (10 μM) on the fluorescence intensity of APA-Zn was detected within 30 min. Result in Figure S9 showed that addition of EDTA-2Na decreased the fluorescence intensity, thus the amount of added EDTA-2Na for capturing free Zn^{2+} should be dependent on the dissolution of nZnO. Addition of EDTA-2Na inhibited the extracellular dissolution of nZnO within 24 h, indicating this chelator was suitable for capturing extracellularly released Zn^{2+} and could verify that the 24 h-internalized Zn is all in nanoform.

Table S1. Diameter of ZnO NPs determined by TEM.

	Diameter (nm)	Hydrodynamic diameter (nm)
Medium	/	16.43±0.31 (0.280±0.06)
ZnO-11	11.30±1.19	219.47±4.74 (0.29±0.14)
ZnO-20	19.71±1.97	201.5±9.31 (0.31±0.009)
Nanorod	4-5 (aspect ratio)	176.1±7.49 (0.41±0.03)
APTES-ZnO	8.79±0.83	448.9±16.5 (0.41±0.04)
Citrate-ZnO	11.27±1.12	120.8±3.80 (0.43±0.02)

Data shown as Mean±SD (PDI±SD).

Table S2. Zeta potential of nZnO in water under different pH values.

	3	7	11
ZnO-11	-13.74±1.66	28.07±1.46	-19.46±1.88
ZnO-20	-16.18±1.02	23.18±1.56	-23.97±1.99
Nanorod	-15.90±0.67	16.02±1.45	-22.46±1.70
APTES-ZnO	8.14±2.40	36.63±1.17	4.85±0.82
Citrate-ZnO	-14.81±4.50	-13.52±0.45	-23.88±0.37

Data shown as Mean±SD.

Table S3. Contents of total Zn (pg/cell).

	7 h	24 h
ZnO-11	6.92±0.82	4.23±0.43
ZnO-20	9.92±1.87	7.98±3.23
Nanorod	22.4±4.22	13.2±3.12
APTES-ZnO	6.55±2.32	6.11±0.84
Citrate-ZnO	3.39±0.73	2.72±0.45
EDTA+ZnO-11	16.4±1.46	11.9±4.89
EDTA+ZnO-20	24.4±9.66	4.25±1.24
EDTA+Nanorod	57.5±22.8	42.8±12.13
EDTA+APTES-ZnO	53.0±20.5	11.2±2.28
EDTA+Citrate-ZnO	31.1±10.16	3.87±0.18

Data shown as Mean±SD.

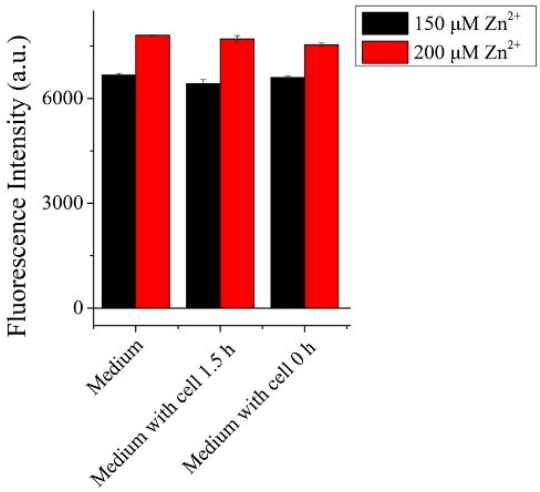

Figure S1. Determination of internalized/adsorbed APA-Zn^{2+} in the 1.5 h dyeing period.

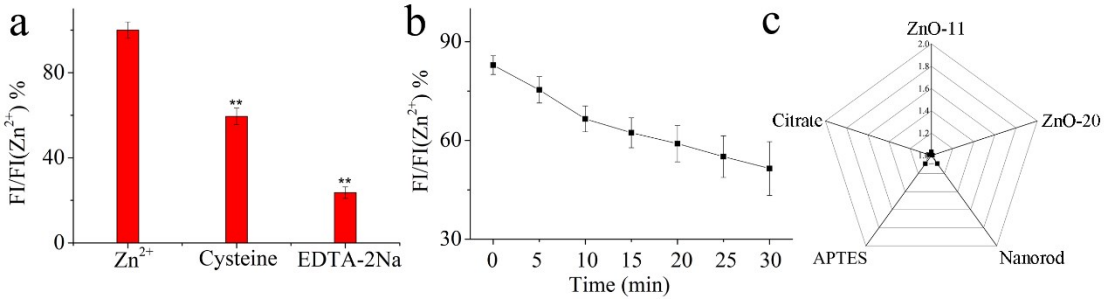

Figure S2. EDTA-2Na as a sensitive molecule for capturing Zn^{2+}. a, Inhibition of cysteine and EDTA-2Na on combination of Zn^{2+} with APA. b, Long term effect of EDTA-2Na on combination of Zn^{2+} with APA. c, [Zn^{2+}] detected by APA after 24 h exposure of EDTA-2Na and nZnO.

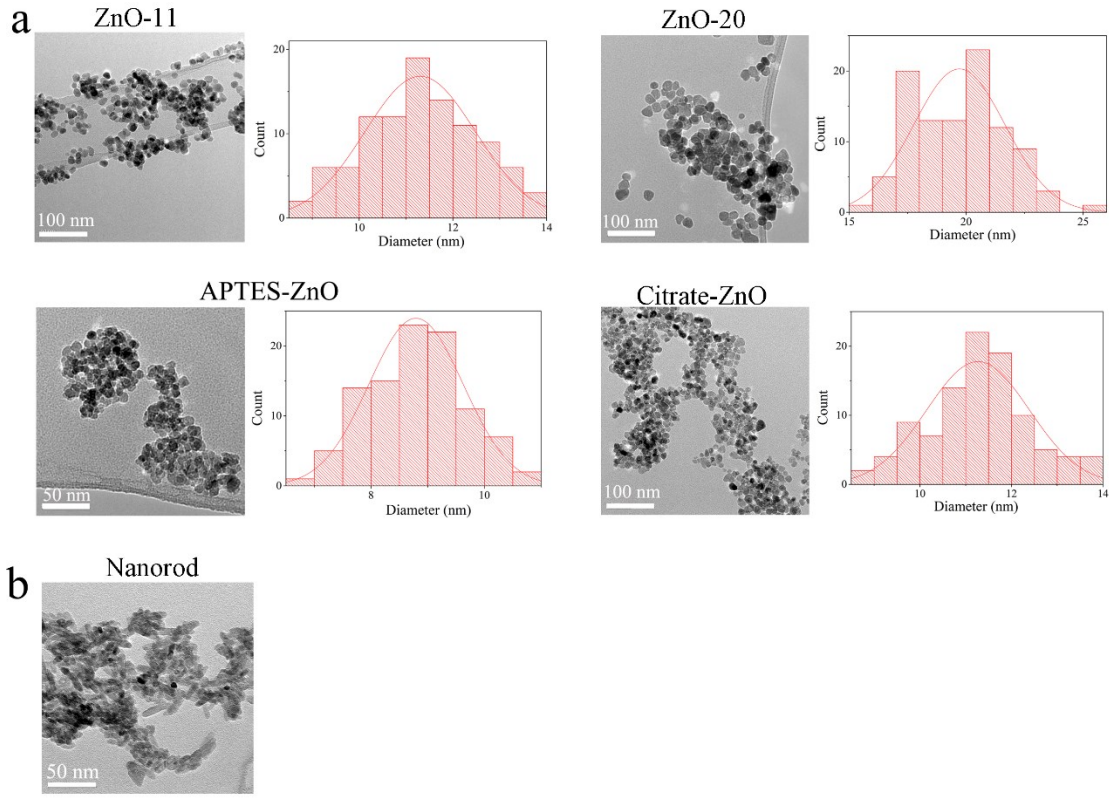

Figure S3. Nano ZnO characterized by TEM. a, Morphology and size distribution of nanoparticles. b, Morphology of nanorods.

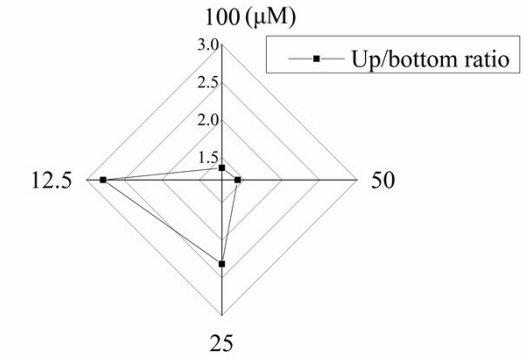

Figure S4. Ultrafiltration efficiency of different [Zn^{2+}] in cell culture medium. The "12.5", "25", "50" and "100" indicate the concentration of Zn^{2+} in the top part of an ultrafiltration tube. The "1.5", "2.0", "2.5" and "3.0" indicate the ratio of [Zn^{2+}] in the top part to [Zn^{2+}] in the bottom part in an ultrafiltration tube.

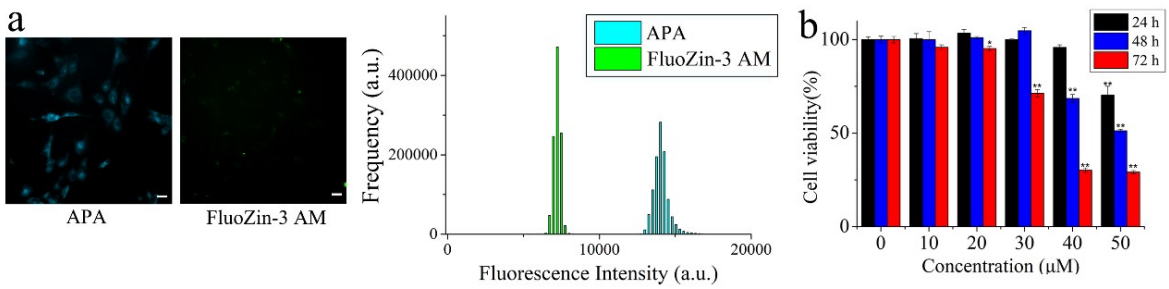

Figure S5. a, Fluorescence images of cells treated with Zn^{2+} and fluorescence probes (10 μM, AIE probe-APA and commercial probe-FluoZin-3 AM). b, Biocompatibility of APA.

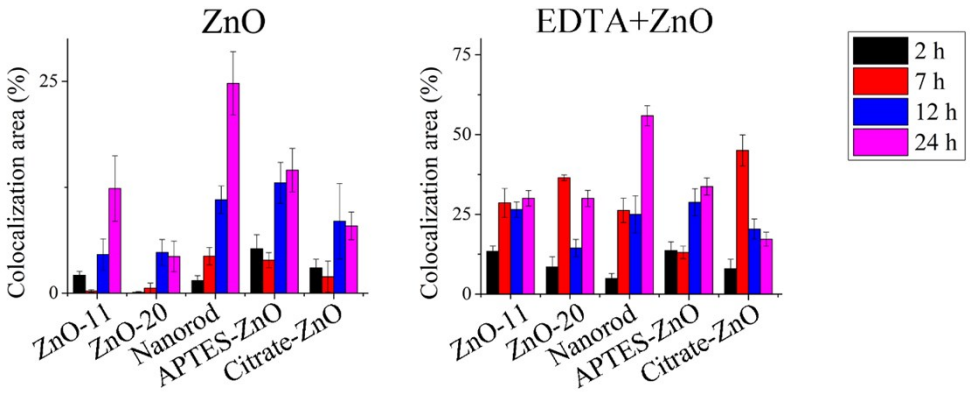

Figure S6. Kinetics of colocalization area which represents colocalization of labile Zn^{2+} and mitochondria within 24 h.

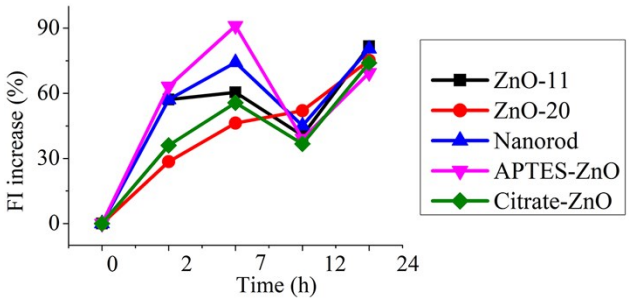

Figure S7. Kinetics of ROS overproduction within 24 h.

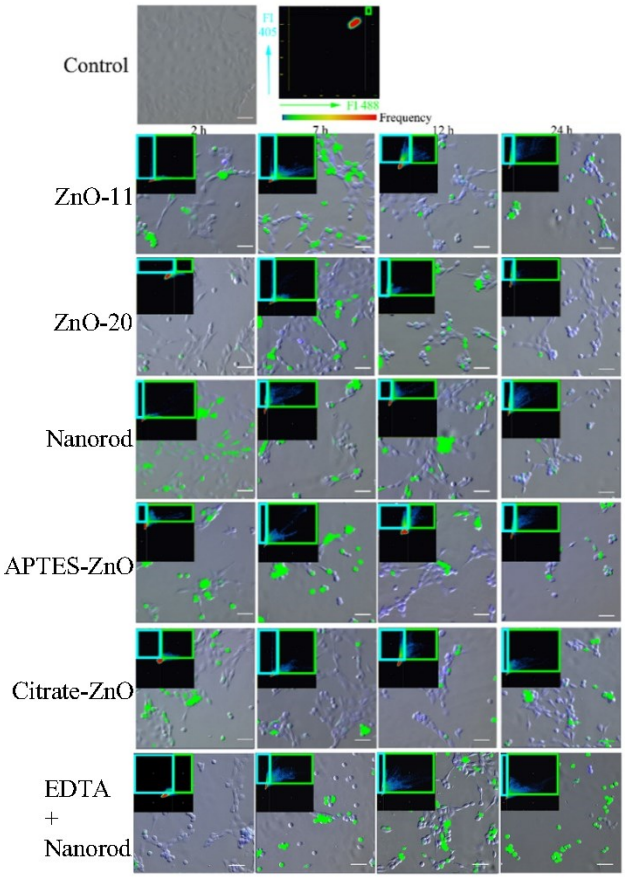

Figure S8. Colocalization analysis. Green parts displayed on cells and circled by green squares represent the colocalization area of labile Zn^{2+} and produced ROS. Cyan squares circle pixels with fluorescence increase of labile Zn^{2+} but no ROS increase. Scale bar: 20 μm.

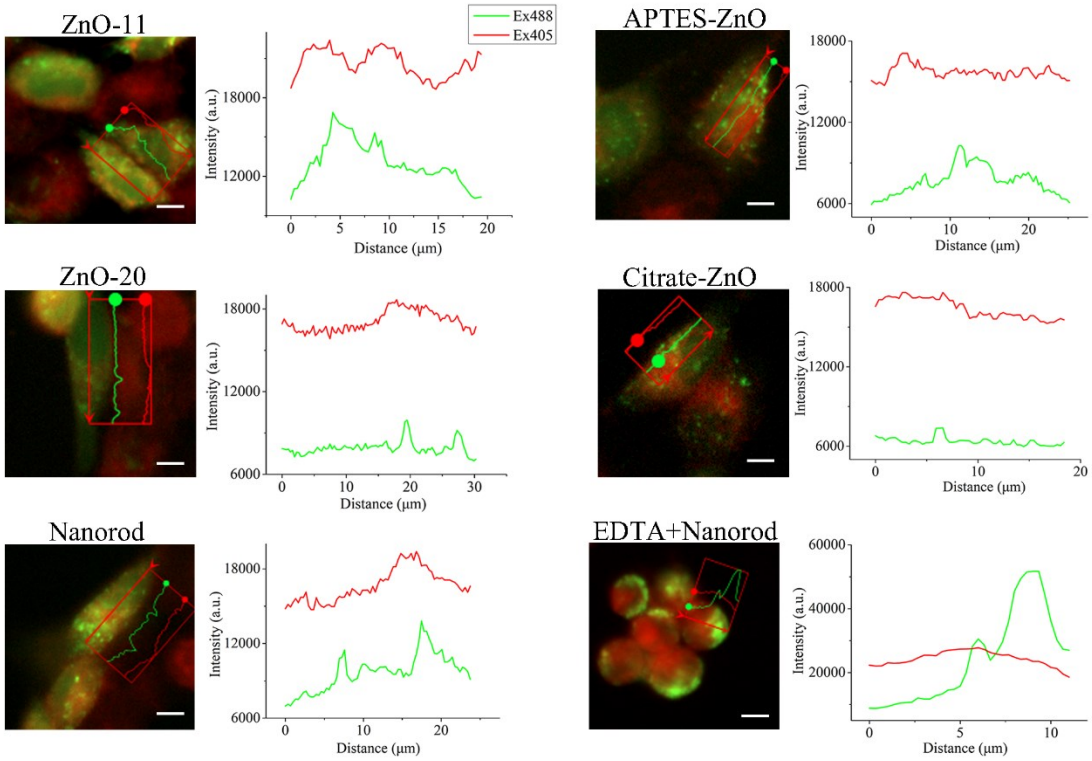

Figure S9. Relationship between the produced ROS and intracellular labile Zn^{2+} after 7 h-treatment (24 h for nanorod group with EDTA addition). Scale bar: 5 μm.