Analysis of Copper Nanoparticles Toxicity Based on a Stress-responsive Bacterial Biosensor Array

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Figure S1. Schematic diagram of the stress-responsive bacterial biosensor. The toxic agents-caused cellular stress induces the production of the related stress-inducible activator protein, and then this protein activates the stress-responsive promoter. Both the protein and the promoter belong to one of the global regulatory systems. The same stress-responsive promoter which is fused to the reporter gene in the recombinant plasmid is also activated by the stress-inducible activator protein. With this activated promoter, the transcription of the reporter gene is turned on, and the corresponding gene product as signal can be detected. In this way, the biosensing bacterium responds to these toxic agents and produces easily detectable signal, such as fluorescence and luminescence.



Figure S2. Luminescence of (A) DPD2511, (B) DPD2794, (C) DPD2540 and (D) TV1061 in the presence and absence of the representative stress-induce compounds with different pre-culture times. The y axis is the maximum luminescence emitted from the induced or non-induced strain. DPD2511 was exposed to 30 mg/L hydrogen peroxide, DPD2794 to 2 mg/L mitomycin, DPD2540 to 100 mg/L phenol, and TV1061 to 3% ethanol, respectively. Each data point is an average of three individual replicates, with error bars denoting the standard deviation.



Figure S3. Luminescence spectra of (A) DPD2511, (B) DPD2794, (C) DPD2540 and (D)

TV1061 responses to different concentrations of CuNPs.



Figure S4. Detailed core-level XPS spectra of copper (2p) and oxygen (1s) for CuNPs and CuMPs. The broad Cu $2p_{3/2}$ peak has been deconvoluted into two peaks, a and b for CuNPs, and e and f for CuMPs. The peaks a and e accord very well with the reported peak of metal Cu,¹ and b and f are related to CuO,² respectively. The oxygen peak was also resolved into two peaks which are marked as peaks c and d for CuNPs, and g and h for CuMPs. The peaks of lowest binding energy (d and g) were assigned to oxygen (O²⁻) in CuO, and the other peaks (c and h) to absorbed hydroxyl species on the oxide surface.^{3, 4}



Figure S5. Growth curves (OD_{600}) of strain RFM443 in LB medium in the presence of different concentrations of (A) CuNPs, (B) Cu²⁺, (C) CuMPs.



Figure S6. Growth curves (OD_{600}) of strain RFM443 in LB medium in the presence of CuNPs alone, or together with SOD, CAT, and THPTA, respectively. Strain RFM443 grew in LB medium worked as a control.



Figure S7. (A) Response of strain DPD2511 to various concentrations of H_2O_2 . (B) Comparison of responses of stress-specific recombinant bioluminescent bacteria to CuNPs and to H_2O_2 .



Figure S8. Dissolution kinetics of CuNPs and CuMPs in LB medium.



Figure S9. Responses of stress-respective biosensing bacteria to 80 mg/L CuNPs, the supernatant of 80 mg/L CuNPs, 1.25 mM THPTA, 200 U/mL CAT, and 200 U/mL SOD, respectively.



Figure S10. Hydrodynamic size and Zeta potential of CuNPs in LB medium determined by DLS and electrophoretic mobility of CuNPs.

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