Supporting information

Surface Topography of Nanoplastics Modulates Their Internalization and Toxicity in Liver Cells

Neng Yan[1], Yuhuai Wang[1], Tin Yan Wong[2], Yubing Hu[3], Huo Xu[4], Parodi Alessandro[5], Ke Pan[4], Jingjing Liu,[6] Henry Lam[2], Ben Zhong Tang[7*], Jianbo Shi[1*]

[1]*State Environmental Protection Key Laboratory of Source Apportionment and Control of Aquatic Pollution, School of Environmental Studies, China University of Geosciences, Wuhan 430074, China*
[2]*Department of Chemical and Biological Engineering, The Hong Kong University of Science and Technology, Kowloon, Hong Kong 999077, China*
[3]*Department of Chemical Engineering, Imperial College London, London SW7 2BU, U.K*
[4]*SZU-HKUST Joint PhD Program in Marine Environmental Science, Shenzhen University, Shenzhen 518060, Guangdong Province, China*
[5]*Scientific Center for Translation Medicine, Sirius University of Science and Technology, 354340 Sochi, Russia*
[6] *Department of Biomedical Engineering, Nanjing University of Aeronautics and Astronautics, Nanjing 210016, China*
[7]*Shenzhen Institute of Aggregate Science and Technology, School of Science and Engineering, The Chinese University of Hong Kong, Shenzhen, Guangdong 518172, China*

*Corresponding author, Email: tangbenz@cuhk.edu.cn; jbshi@rcees.ac.cn;

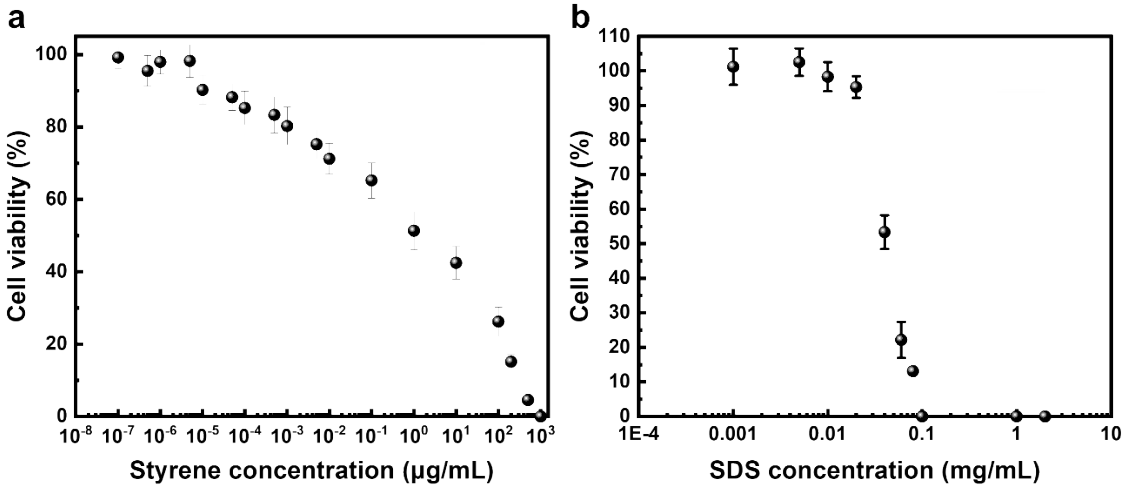

Figure S1. 24 h cytotoxicity test of styrene and SDS towards the L02 cells.

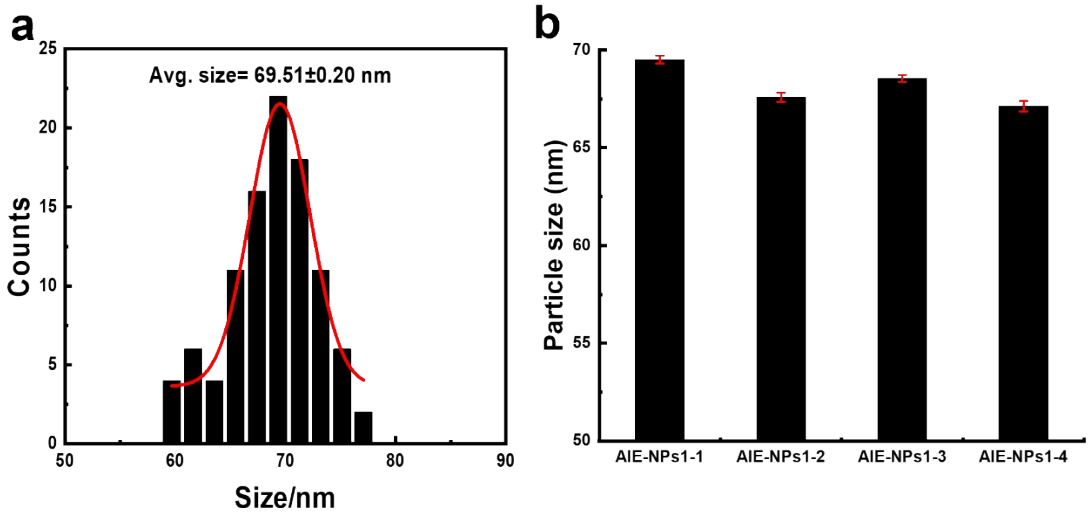

Figure S2. Size distribution of the synthesized AIE-NPs1.

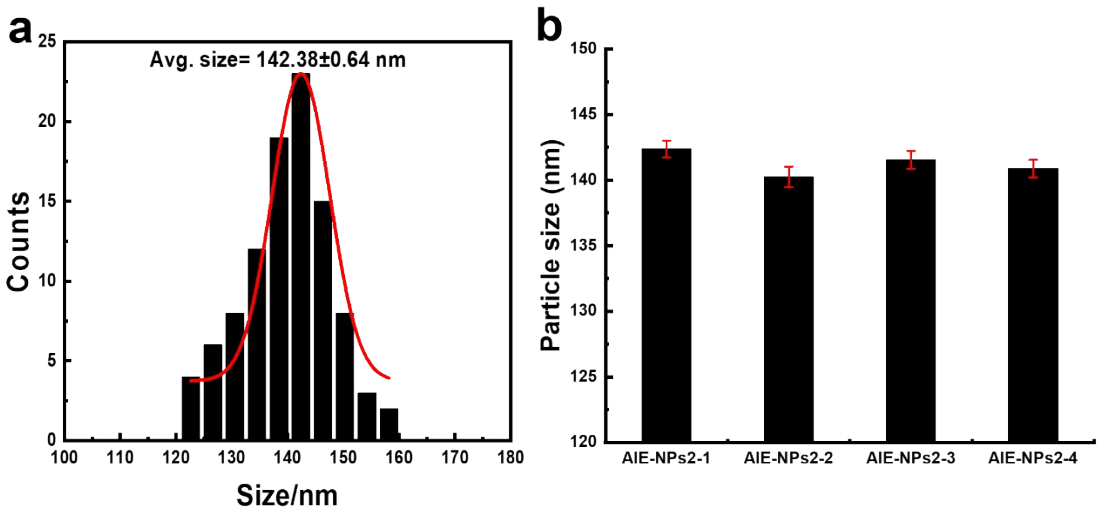

Figure S3. Size distribution of the synthesized AIE-NPs2.

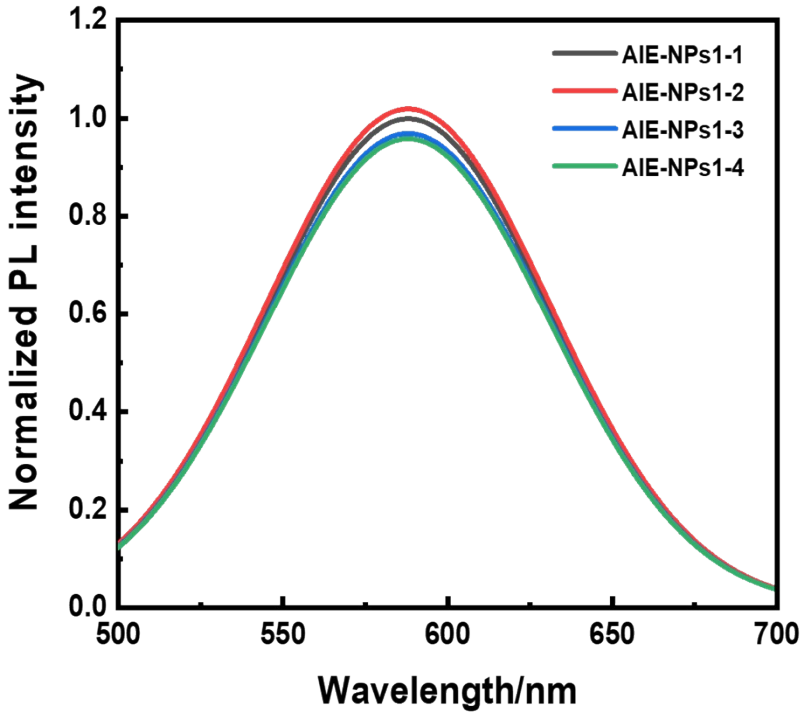

Figure S4. Normalized PL intensity of 10 mg/L of AIE-NPs1.

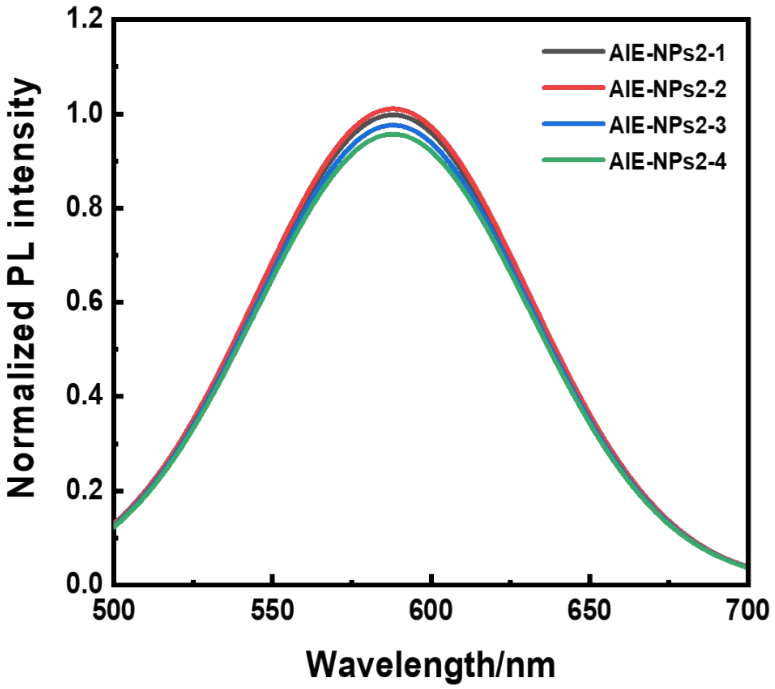

Figure S5. Normalized PL intensity of 10 mg/L of AIE-NPs2.

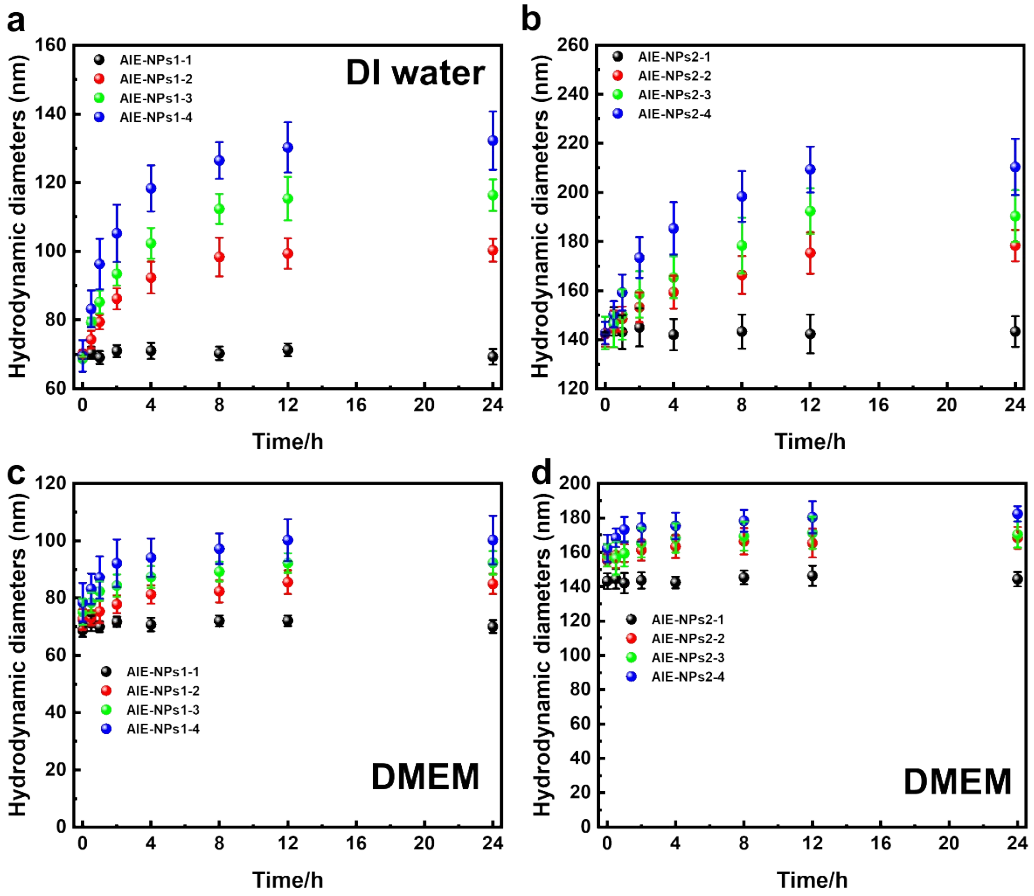

Figure S6. Aggregation kinetics of 10 mg/L of AIE-NPs within 24 h exposures. The hydrodynamic diameter changes of AIE-NPs1 (a) and AIE-NPs2 (b) in the DI water and the hydrodynamic diameter changes of AIE-NPs1 (c) and AIE-NPs2 (d) in the DMEM medium, respectively.

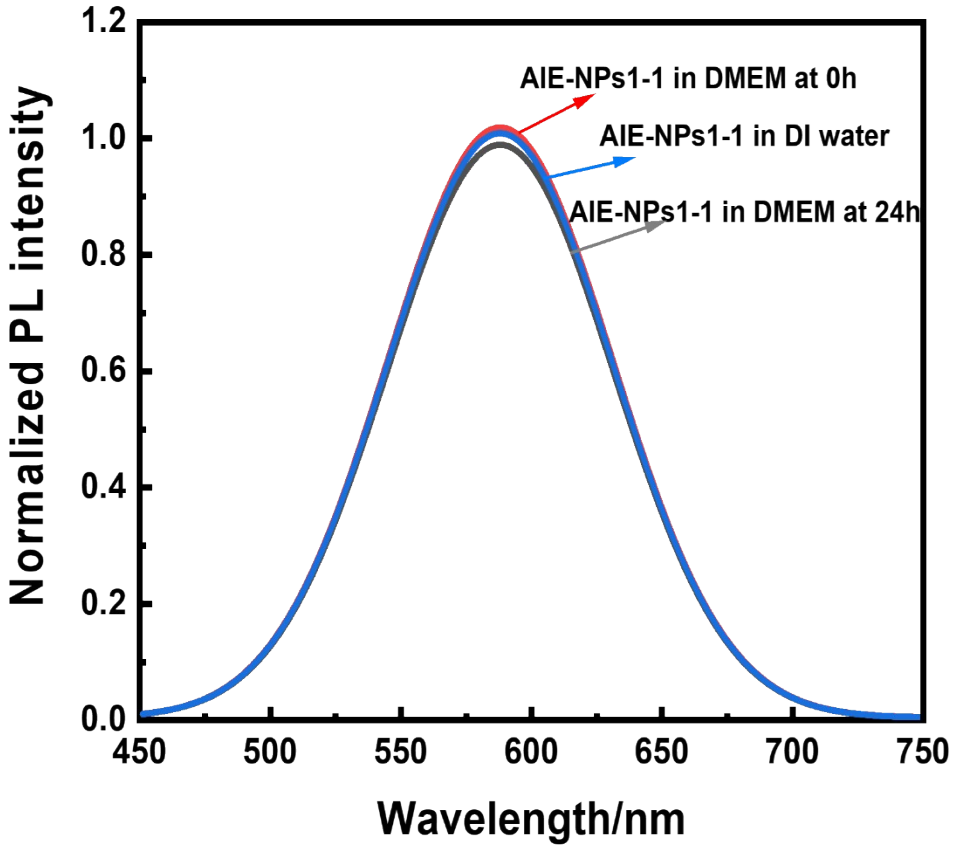

Figure S7. Evaluating the photostability of 10 mg/L of the AIE-NPs1-1 in the DMEM medium after 24 h exposures.

Table S1. The size and zeta potential of the synthesized AIE-NPs in cell culture medium.

Nanoplastics	Size distribution	Zeta potential
AIE-NPs1-1	68.75±2.23	-32.58±1.27
AIE-NPs1-2	72.55±3.58	-33.25±2.03
AIE-NPs1-3	75.23±4.55	-30.29±3.01
AIE-NPs1-4	78.44±6.77	-36.25±2.88
AIE-NPs2-1	143.23±4.56	-34.22±2.53
AIE-NPs2-2	155.29±3.58	-35.52±2.14
AIE-NPs2-3	158.43±5.68	-34.95±2.57
AIE-NPs2-4	162.23±7.98	-36.83±1.95

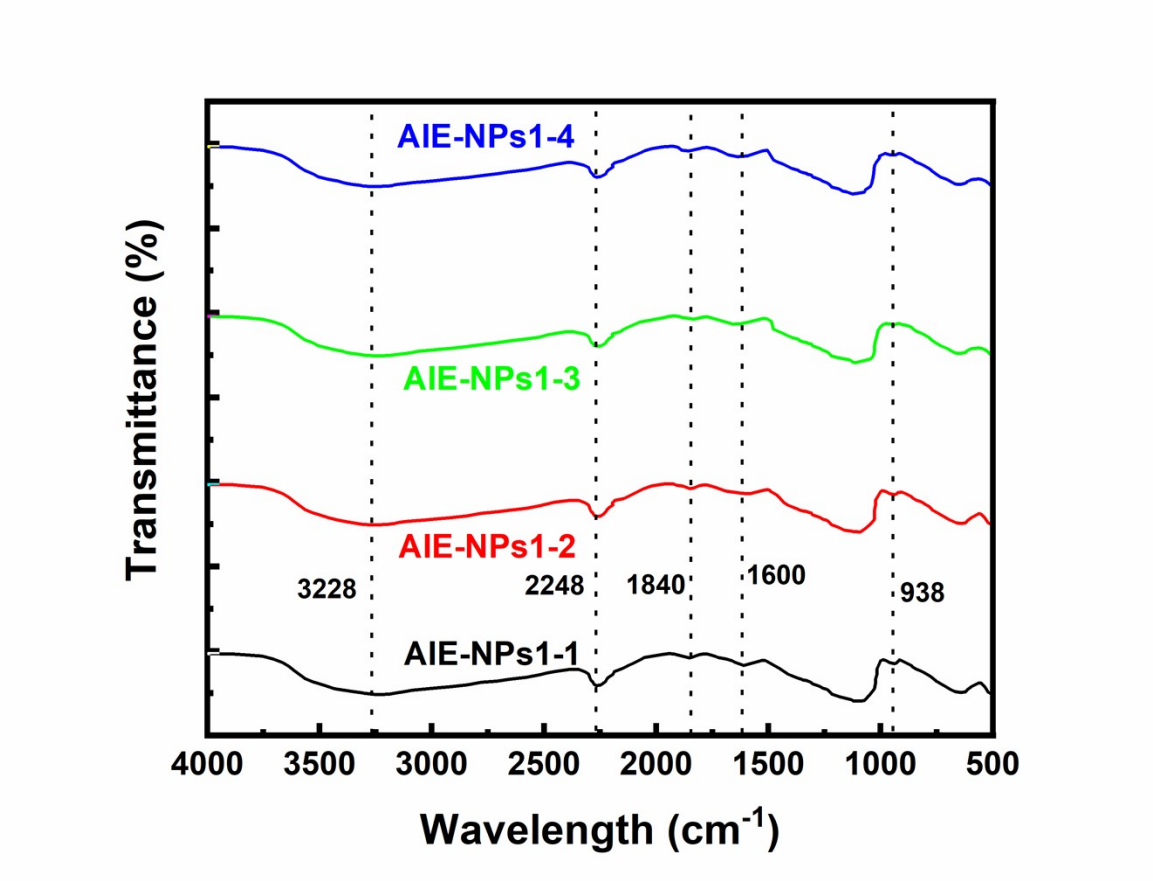

Figure S8. Infrared spectra of AIE-NPs1 after HDS treatment for different durations.

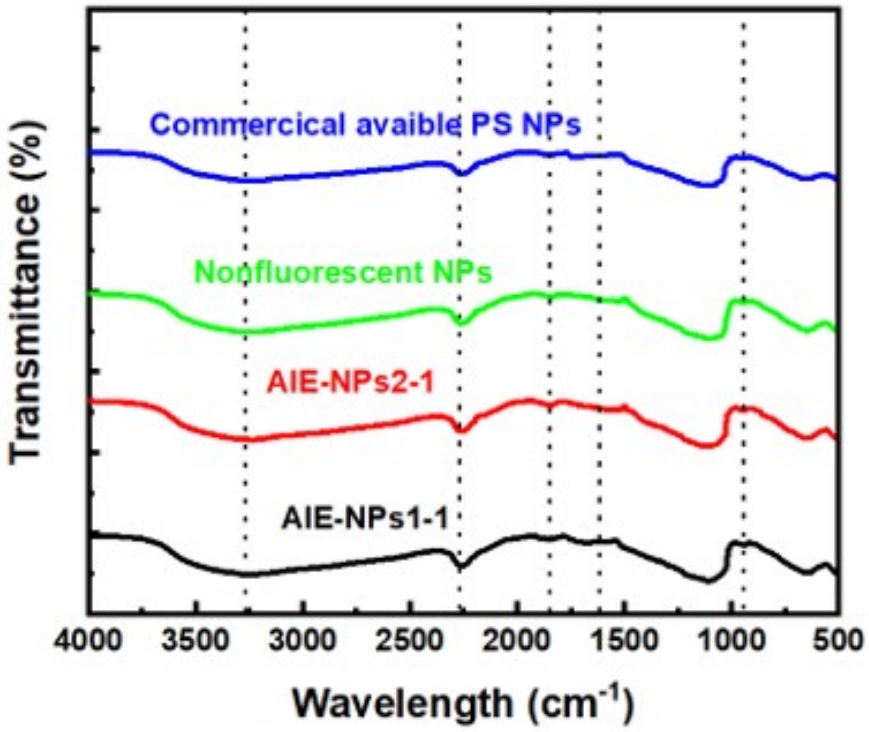

Figure S9. FTIR spectra of PS NPs, the synthesized nonfluorescent PS NPs, synthesized AIE-NPs1-1 and AIE-NPs2-1.

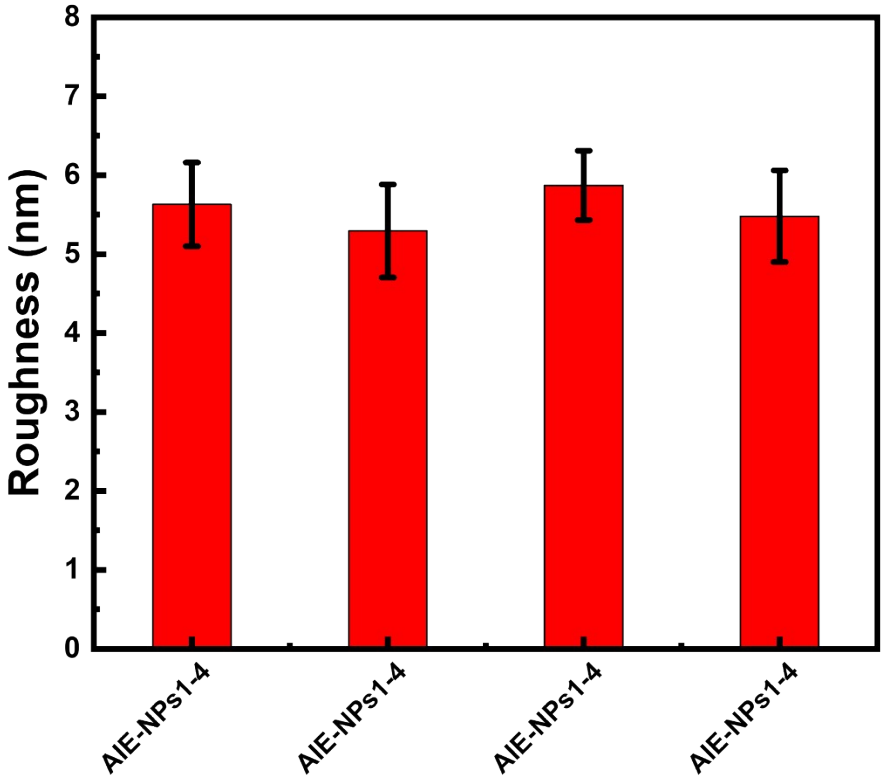

Figure S10. The surface roughness measurement of the obtained 10 mg/L of AIE-NPs1-4 (by treating the AIE-NPs1 in the HDS environment at different dates). There were three replicates in each treatment. No significant difference has been identified in four treatments using one-way ANOVA (P=0.62, >0.5, no significant difference).

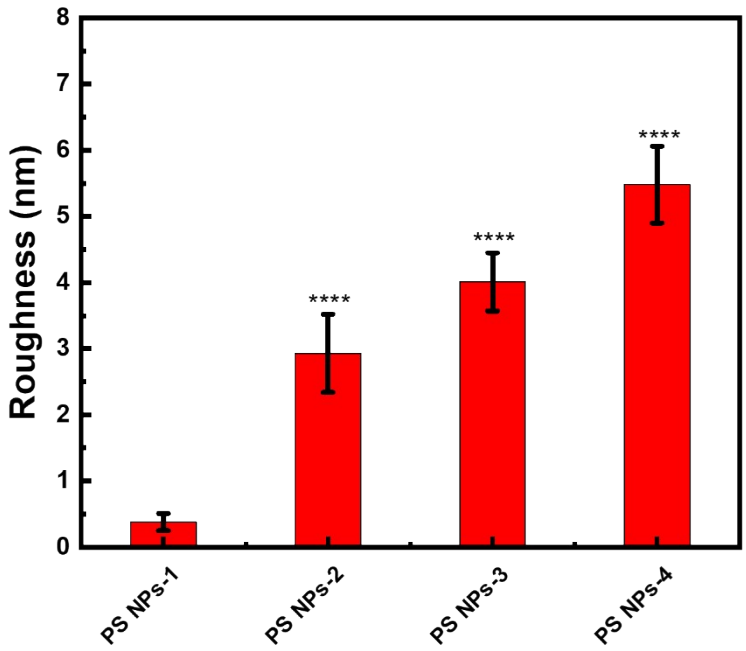

Figure S11. Surface roughness measurement of commercially available 70 nm sized PS NPs that are being exposed to HDS environment for different durations (as AIE-NPs1).

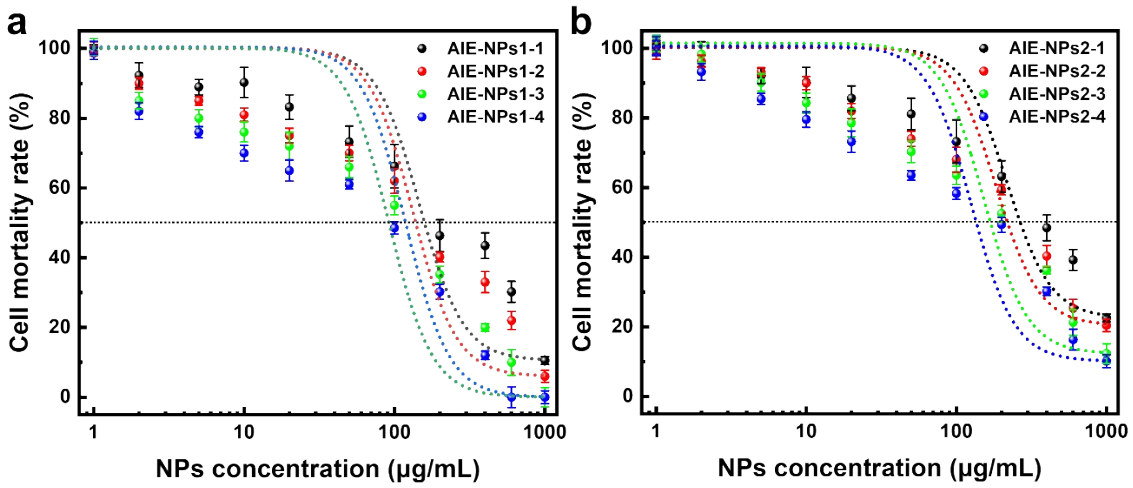

Figure S12. Cytotoxicity of AIE-NPs1 (a) and AIE-NPs2 (b) after 24 h exposure.

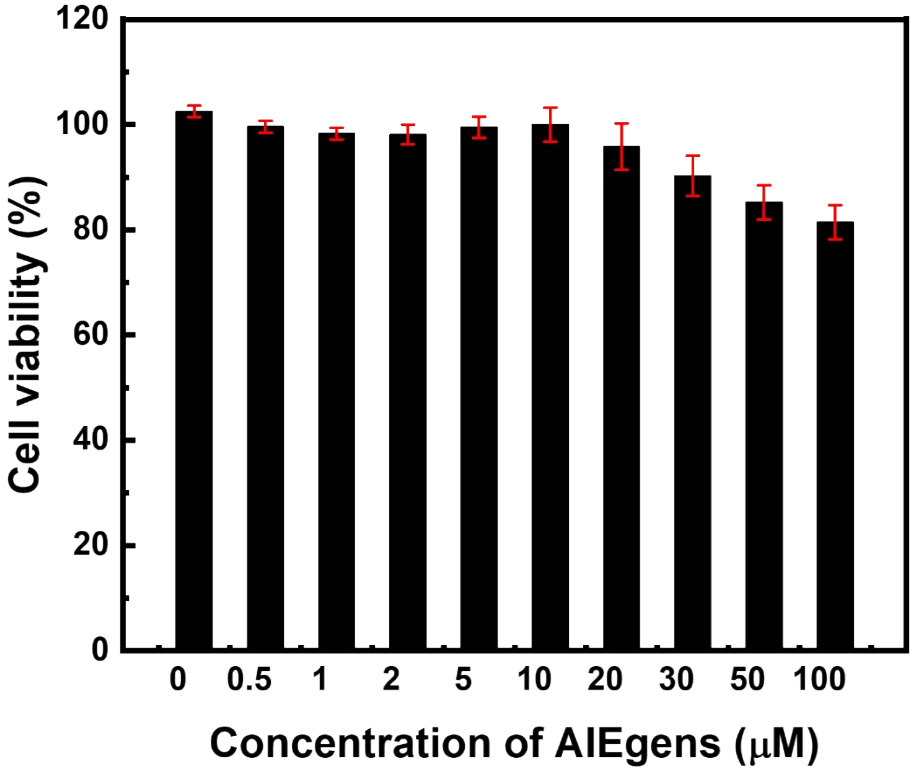

Figure S13. 24 h cytotoxicity test of AIEgens towards the L02 cells.

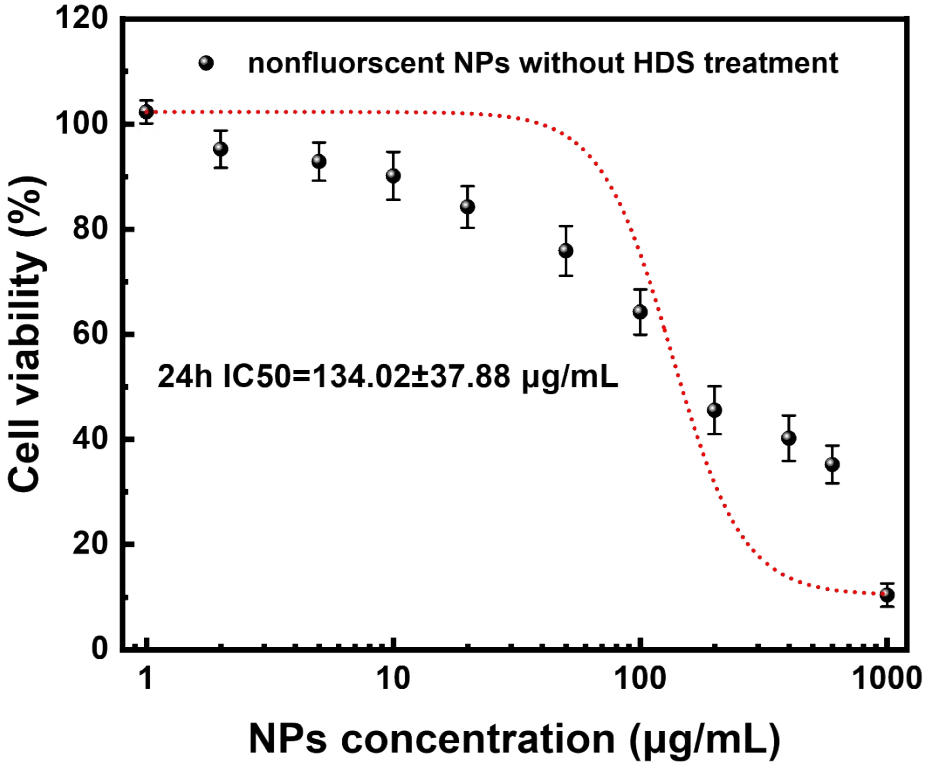

Figure S14. Cytotoxicity of the synthesized nonfluorescent nanoplastics (the same synthesis processes as the AIE-NPs1 but without HDS treatment) after 24 h exposure. The measured 24h IC50 was 134.02±37.88 mg/L.

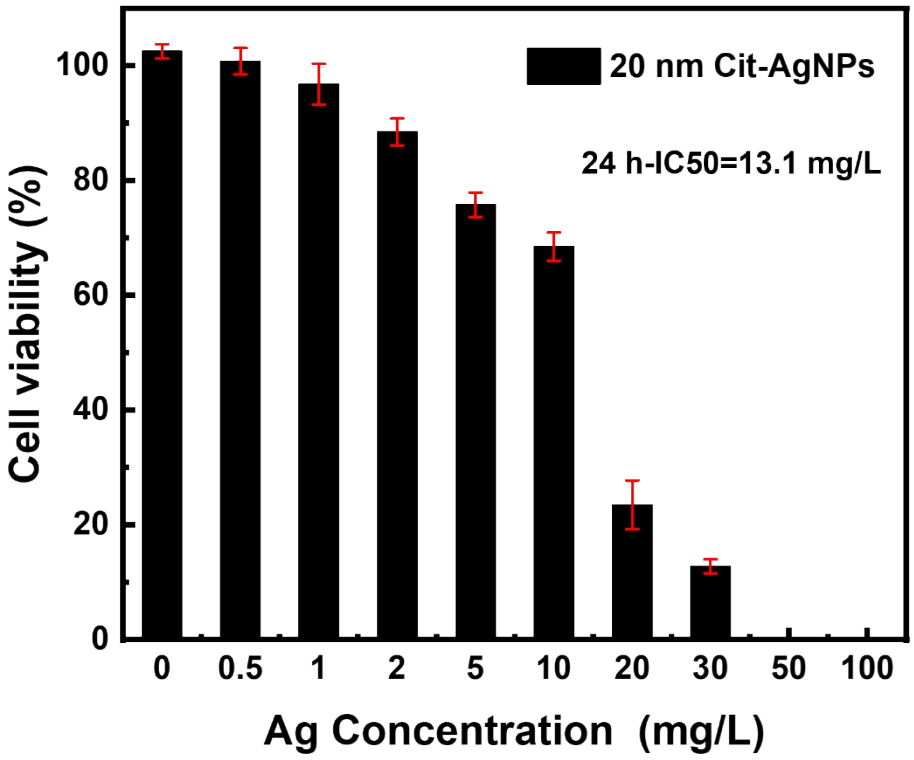

Figure S15. 24 h cytotoxicity test of 20 nm citrate coated AgNPs towards the L02 cells.

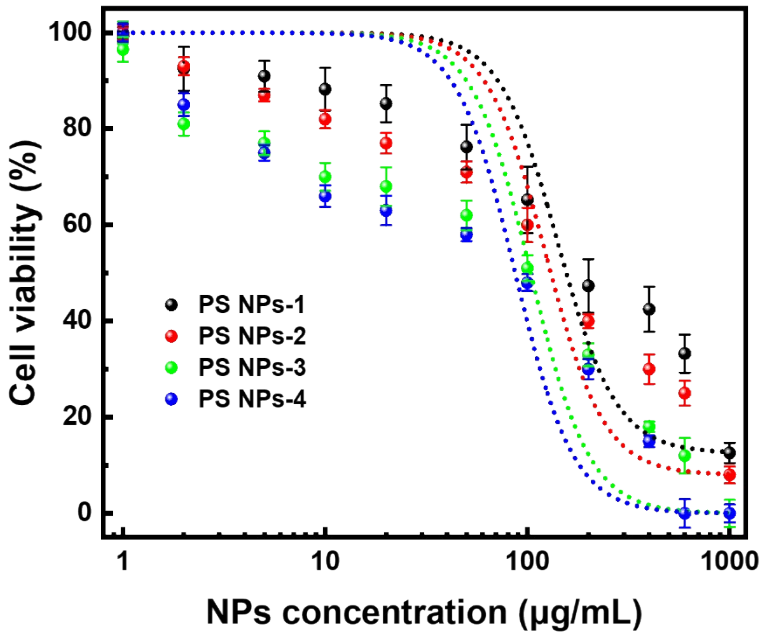

Figure S16. Cytotocixity of HDS treated commercially available 70 nm sized PS NPs after 24 h exposure.

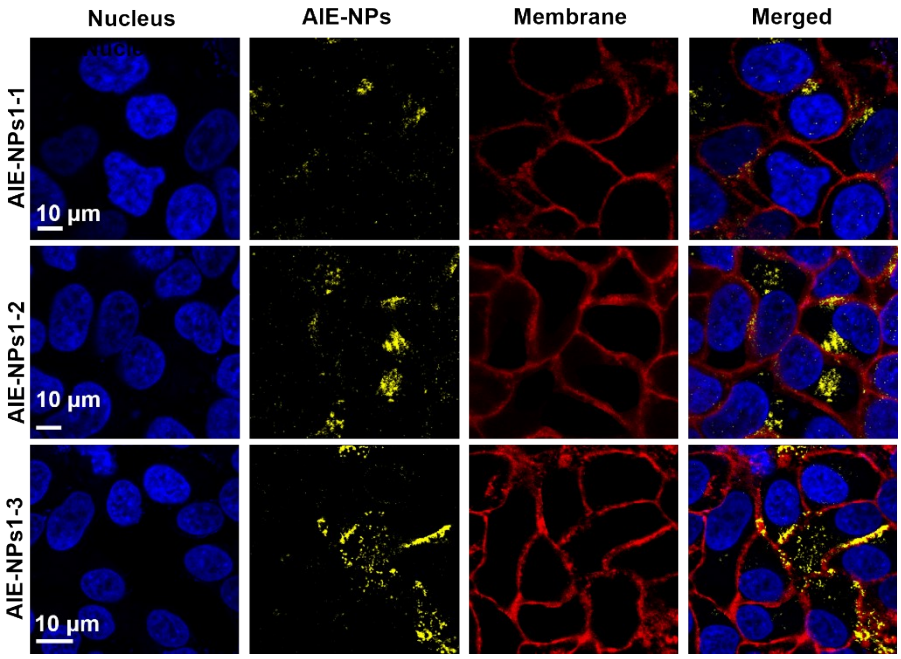

Figure S17. The intracellular patterns of AIE-NPs1-1 to AIE-NPs1-3.

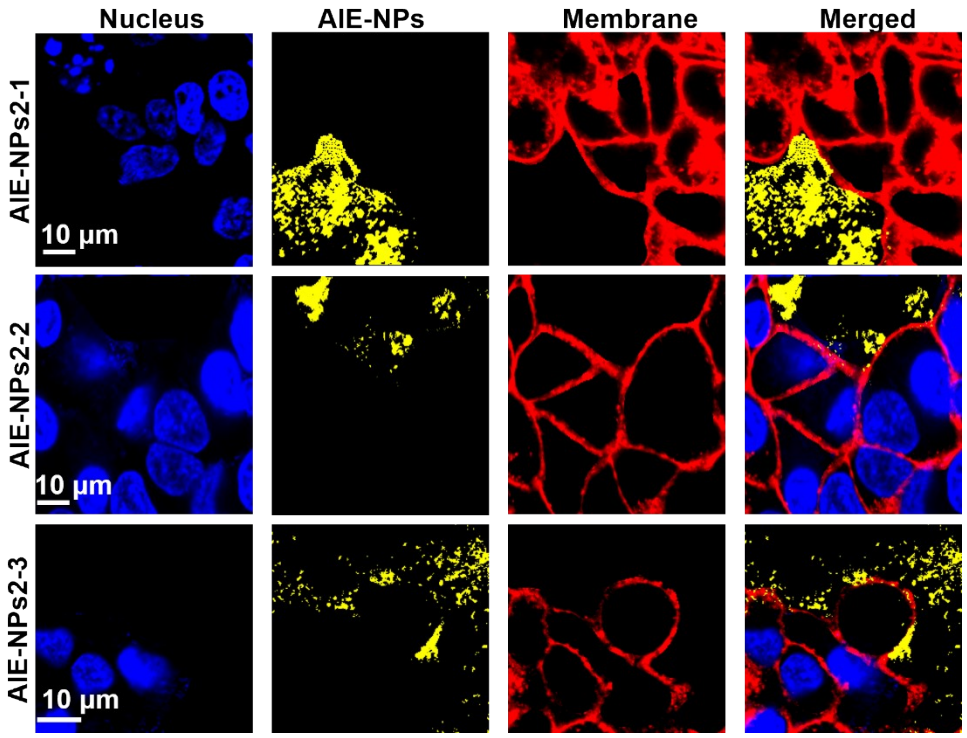

Figure S18. The intracellular patterns of AIE-NPs2-1 to AIE-NPs2-3.

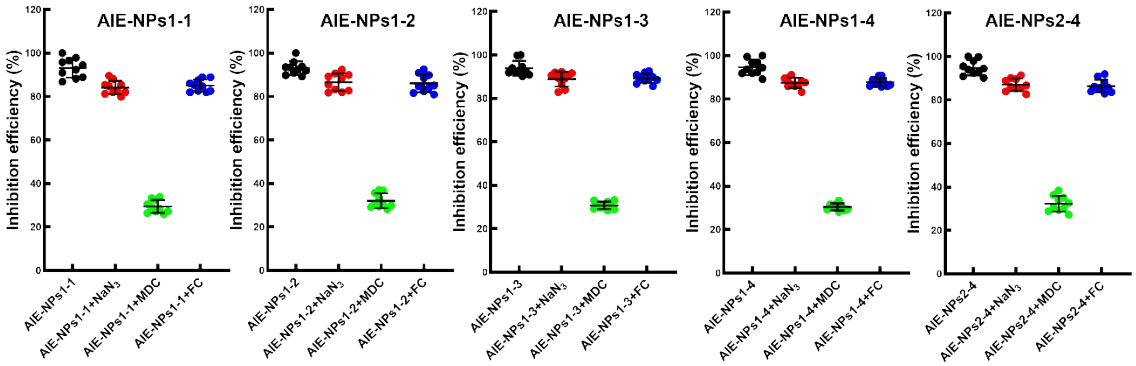

Figure S19. Impact of different endocytosis inhibitors (NaN_3, MDC and FC) on the cellular accumulation of AIE-NPs1-1 to AIE-NPs 1-4 and AIE-NPs2-4. Human hepatocytes were first incubated with sodium azide (NaN_3, 0.25mM), monodansylcadaverine (MDC, 0.2 mM) or filipin complex (FC, 0.07 mM) before the exposure of 10 mg/L AIE-NPs.

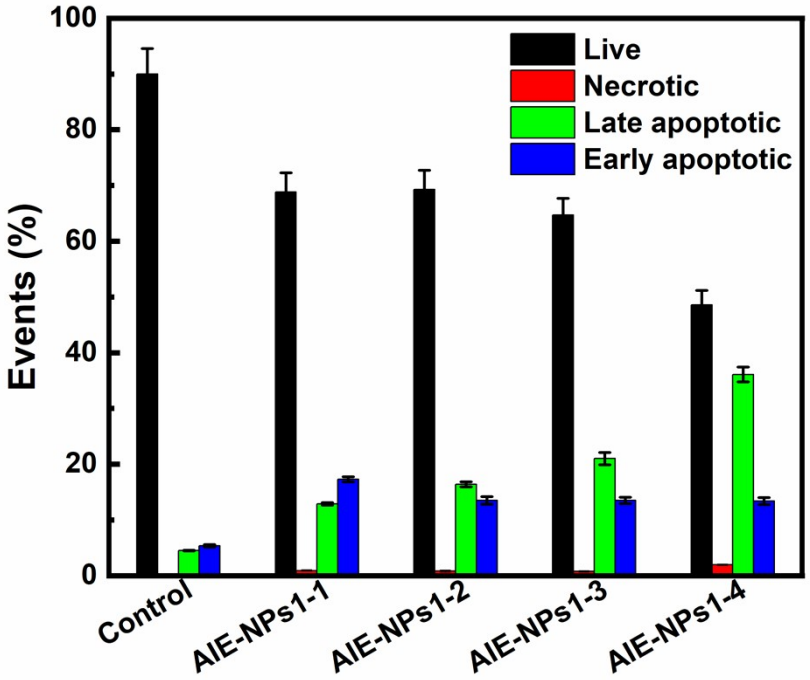

Figure S20. The percentage of cells in live, necrotic, early apoptosis and late apoptosis after the cell being exposed to the AIE-NPs1 or without any AIE-NPs exposure.