Electronic Supplementary Information (ESI)†

A Feasible Self-assembly Near-Infrared Fluorescence Sensor for Acid Phosphatase Detection and Cell Imaging

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Section 1. Characterization

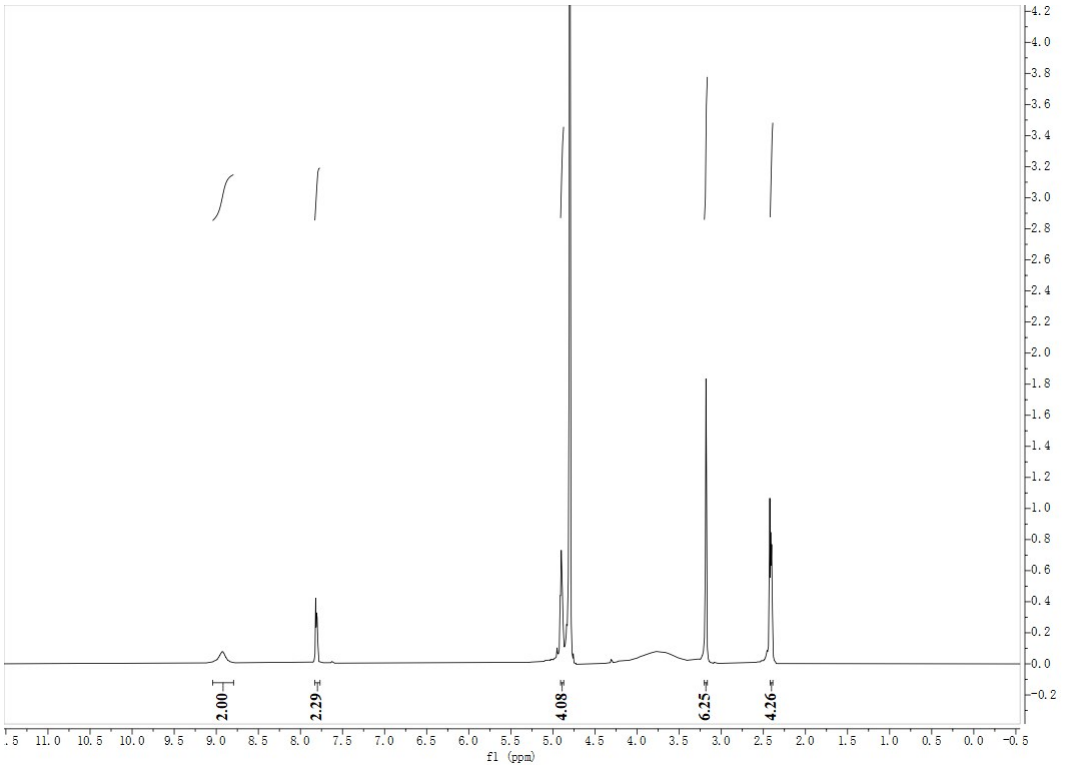

Fig. S1: ^{1}H NMR of Ligand L1.

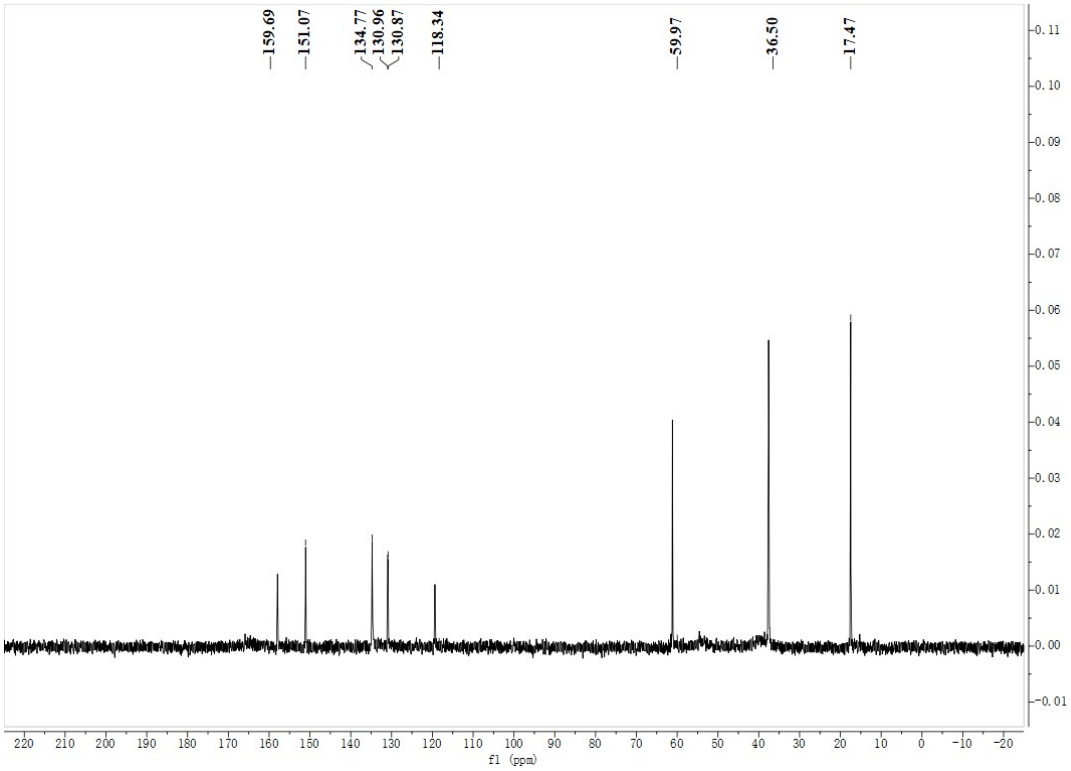

Fig. S2: ^{13}C NMR of Ligand L1.

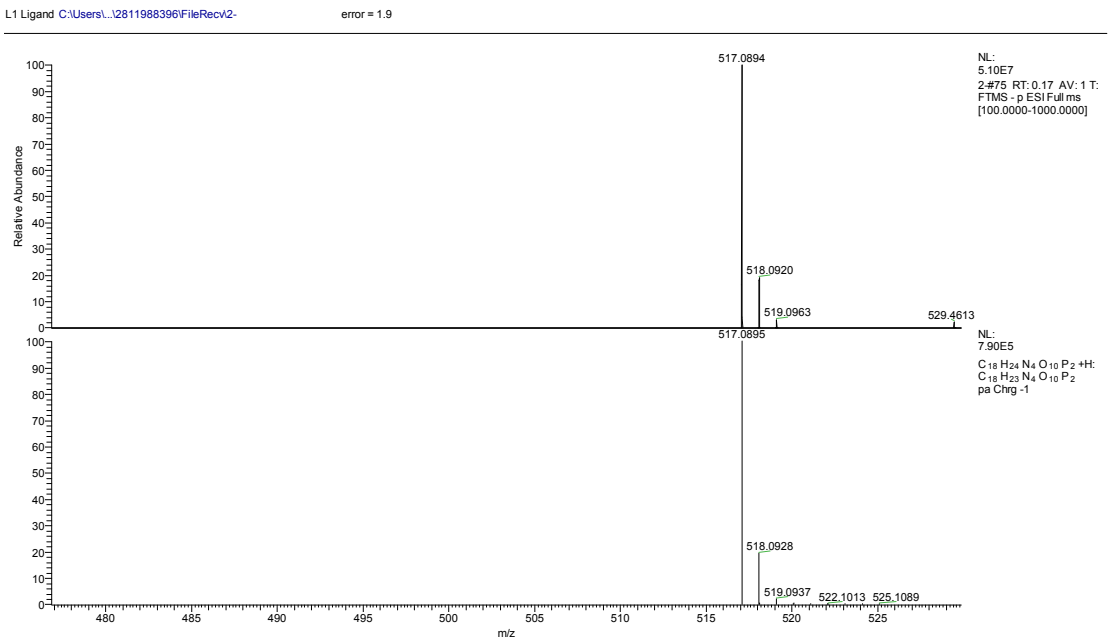

Fig. S3: HRMS of Ligand L1.

Section 2. Feasibility testing

To verify the feasibility of the method, the following solutions were prepared. (I) 10.0 mM aqueous solution of PLP; (II) 0.5 mM aqueous solution of NR; (III) The mixture containing 20.0 μL of L1@NR-Eu-polymer (3 mg/mL), 20.0 μL of $MgCl_2$ (1.0 mM) and HEPES buffer (10.0 mM, pH 5.0) completed to 200.0 μL. (IV) The mixture containing 20.0 μL of L1@NR-Eu-polymer (3 mg/mL), 4.0 μL of stock solution of ACP (500 mU/mL), 20.0 μL of $MgCl_2$ (1.0 mM) and HEPES buffer completed to 200.0 μL. The fluorescence spectra were collected at room temperature.

The reaction time of enzymatic hydrolysis is optimized. The mixture solutions of (IV) were incubated at 37 °C for different time and the fluorescence intensities were recorded at 373 and 613 nm with the excitation at 280 and 513 nm, respectively.

Section 3. Comparison of different materials

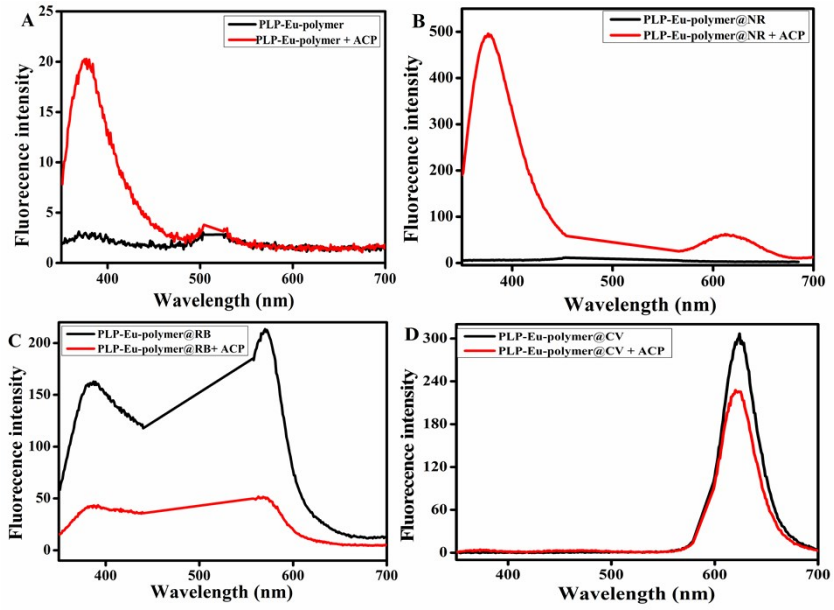

Fig. S4: The emission spectra of PLP-EDA-Eu-Polymer and encapsulated with different dyes (NR, RB, CV) (black line in Fig. A, B, C and D) (0.3 mg/mL); The polymers response to the ACP (10.00 mU/mL) (red line in Fig. A, B, C and D).

Section 4. The optimization of pH

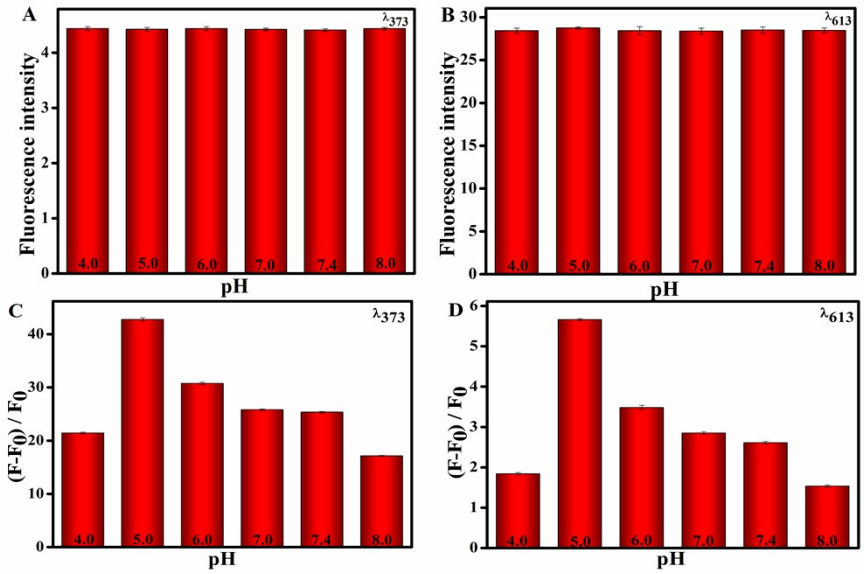

Fig. S5: The stability of the L1@NR-Eu-polymer at different pH (4.0, 5.0, 6.0, 7.0, 7.4, 8.0) of λ_{373} (A) and λ_{613} (B). The optimization of pH of ACP (4.0, 5.0, 6.0, 7.0, 7.4, 8.0) of λ_{373} (C) and λ_{613} (D).

Section 5. Dynamics of L1@NR-Eu-polymer

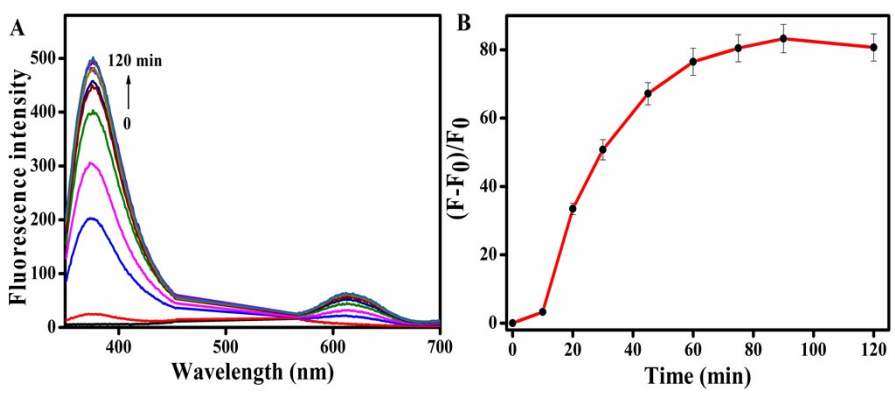

Fig. S6: The activity of ACP (10.00 mU/mL) under different incubation time with L1@NR-Eu-polymer (A); The emission intensities were recorded at 373 nm (B).

Section 6. Cytotoxicity test of L1@NR-Eu-polymer

Living H9C2, RAW 264.7, HepG-2 and HeLa cells were cultured with the Dulbecco's modified Eagle's medium (DMEM) containing 10% FBS (fetal bovine serum) at 37 °C under the atmosphere of 5% CO_2. Cytotoxicity of the L1@NR-Eu-polymer to the cells were performed by the CCK-8 method. The cells were uniformly distributed into 96-well plates with the density of 1.0×10^4 cells per well and incubated for 24 h. Then various concentrations of probes were introduced to the corresponding wells and cultured for another 24 h, extra CCK-8 (10.0 μL) were added to each well and incubated for 1 h afterwards. Finally, microplate reader was applied to record the UV absorbance of the cells. The cell survival rate can be calculated according to the formula $VR = A/A_0 \times 100\%$ (A refers to the absorbance value of the monitoring group, A_0 represents the absorbance value of the control cell).

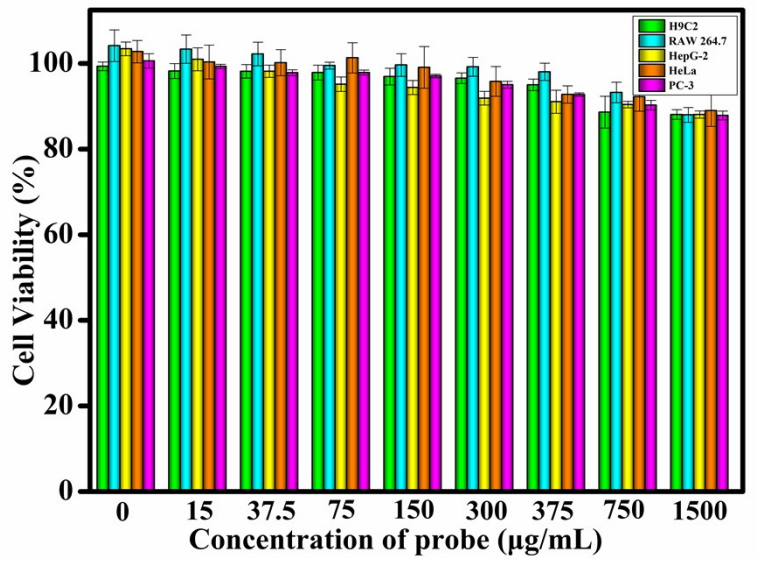

Fig. S7: The investigation of the cell viability of H9C2, RAW 264.7, HepG-2 and HeLa cells after incubated with L1@NR-Eu-polymer of different concentrations (0-1500.0 μg/mL) for 24 h.

Section 7. Cell imaging of L1@NR-Eu-polymer

The RAW 264.7, HepG-2 and HeLa cells were respectively divided into five groups and treated differently for the images. The first group was selected as control without treatment. The second group was incubated with L1@NR-Eu-polymer (0.03 mg/mL) at 37 °C for 1 h, followed by washing with PBS buffer solution before imaging. The third group was incubated with L1@NR-Eu-polymer (0.3 mg/mL) at 37 °C for 1 h. The fourth group was pre-treated with ACP inhibitor Na_3VO_4 (0.20 μM) at 37 °C for 30 min, and then cultured with L1@NR-Eu-polymer (0.3 mg/mL) at 37 °C for 1 h. The last group was pre-treated with ACP inhibitor Na_3VO_4 (2.00 μM) at 37 °C for 30 min, and then cultured with L1@NR-Eu-polymer (0.3 mg/mL) at 37 °C for 1 h. Fluorescence imaging was performed by using confocal fluorescence microscope with the excitation wavelength at 513 nm and the emission wavelength at 550-700 nm.

The living H9C2 were also divided into five groups and treated differently for the images. The first group was selected as control without treatment. The second group was incubated with L1@NR-Eu-polymer (0.03 mg/mL) at 37 °C for 1 h, followed by washing with PBS buffer solution before imaging. The third group was

incubated with L1@NR-Eu-polymer (0.3 mg/mL) at 37 °C for 1 h. The fourth group was cultured with L1@NR-Eu-polymer (0.3 mg/mL) at 37 °C for 2 h. The last group was cultured with L1@NR-Eu-polymer (0.6 mg/mL) at 37 °C for 1 h.

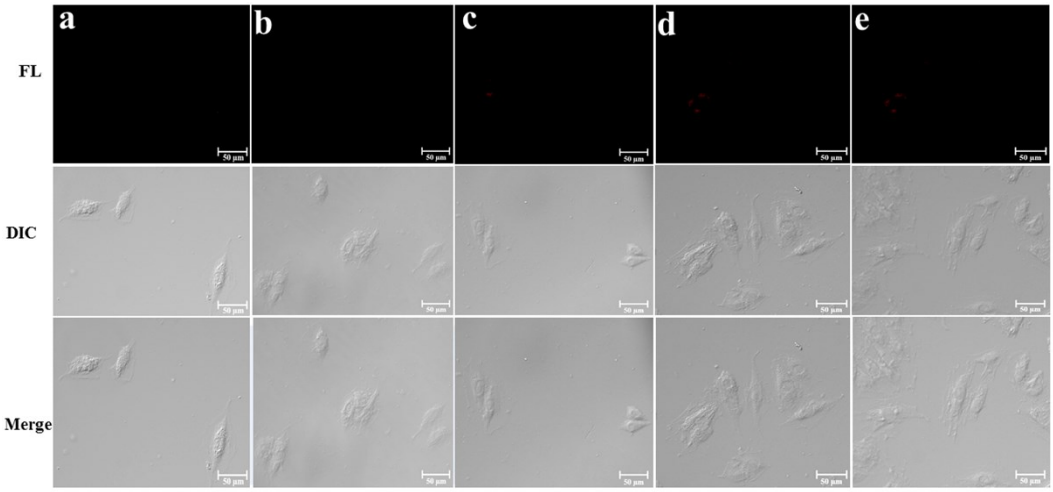

Fig. S8: Representative confocal (FL) and differential interference contrast (DIC) microscopy images of H9C2 cells. Untreated H9C2 control cells (a). H9C2 cells treated with L1@NR-Eu-polymer for 1 h, (0.03 mg/mL) (b) and (0.3 mg/mL) (c). H9C2 cells treated with L1@NR-Eu-polymer (0.3 mg/mL) for 2 h (d). H9C2 cells treated with L1@NR-Eu-polymer (0.6 mg/mL) for 1 h (e).

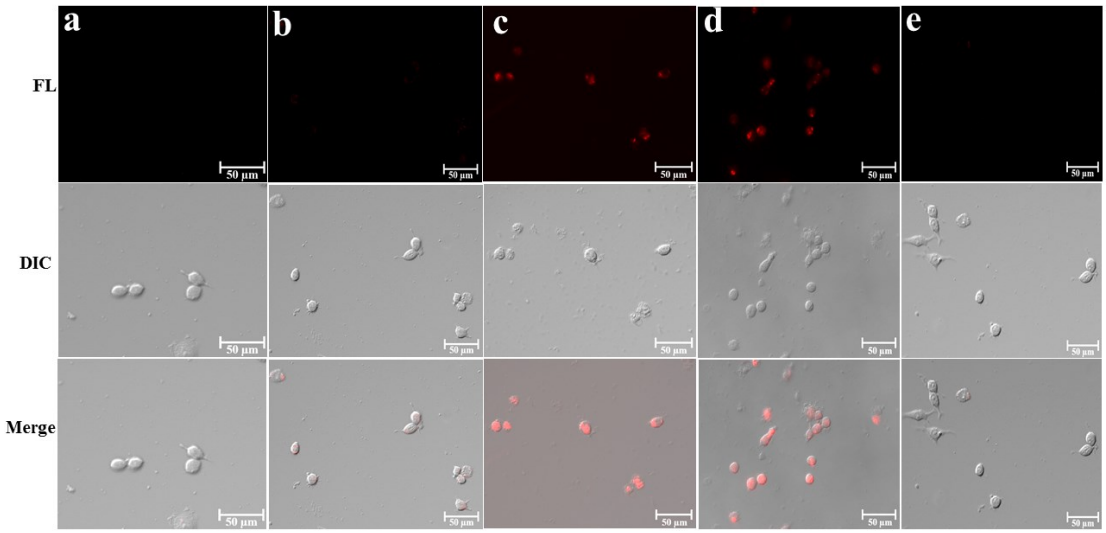

Fig. S9: Representative confocal (FL) and differential interference contrast (DIC) microscopy images of RAW 264.7 cells. RAW 264.7 control cells (untreated) (a). RAW 264.7 cells treated with L1@NR-Eu-polymer for 1 h (0.03 mg/mL) (b) and (0.3 mg/mL) (c). RAW 264.7 cells was pre-treated with ACP inhibitor Na_3VO_4 at 37 °C for 30 min, and then cultured with L1@NR-Eu-polymer (0.3 mg/mL) at 37 °C for 1 h, Na_3VO_4 (0.20 μM) (d) and Na_3VO_4 (2.00 μM) (e).

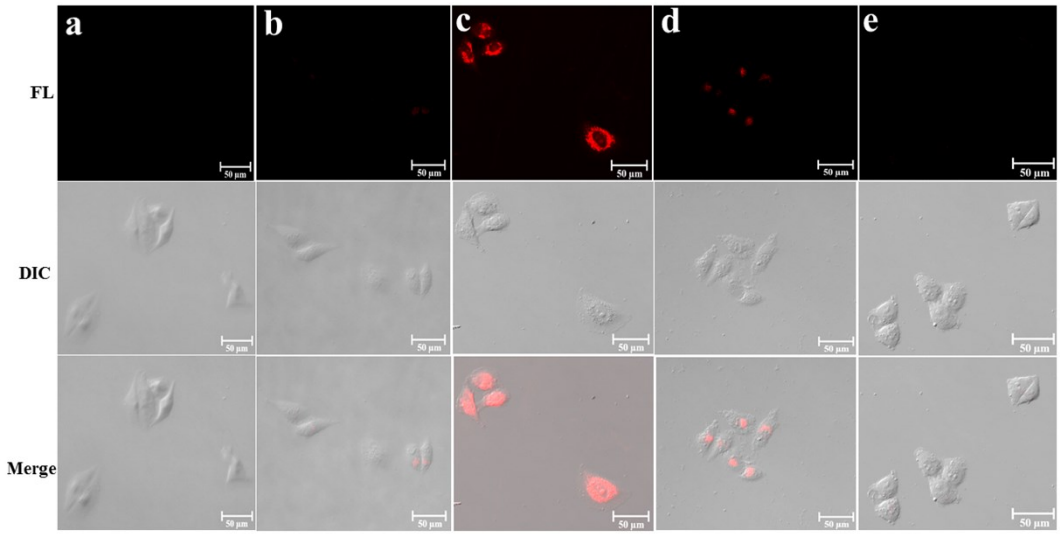

Fig. S10: Representative confocal (FL) and differential interference contrast (DIC) microscopy images of HepG-2 cells. HepG-2 control cells (untreated) (a). HepG-2 cells treated with L1@NR-Eu-polymer for 1 h (0.03 mg/mL) (b) and (0.3 mg/mL) (c). HepG-2 cells was pre-treated with ACP inhibitor Na_3VO_4 at 37 °C for 30 min, and then cultured with L1@NR-Eu-polymer (0.3 mg/mL) at 37 °C for 1 h, Na_3VO_4 (0.20 μM) (d) and Na_3VO_4 (2.00 μM) (e).