Electronic Supplementary Materials (ESI)

ROS-initiated Chemiluminescence-driven Payload Release from Macrocycle-Based Azo-containing Polymer Nanocapsules

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Experimental Procedures

Cell culture.

A12 and RAW 264.7 cell lines were incubated with DMEM and supplemented with 10% FBS at 37 °C in a humidified atmosphere containing 5% CO_2. Cells were sub-cultured regularly using trypsin/EDTA.

Cellular fate study

Simple incubation of Spermine-FITC with LUM@Azo-NCs and subsequent dialysis in water afforded the surface-functionalized NCs, LUM@Azo-NCs@SP-FITC, for studing the cellular fate of NCs. RAW 264.7 cells were incubated with LUM@Azo-NCs@SP-FITC for 24 h, before further incubation with 100 ng/mL of LPS for 0, 12 or 24 h. The fluorescence of FITC was observed via CLSM.

Zebrafish embryos.

Embryo medium: 5 mM NaCl, 0.17 mM KCl, 0.33 mM CaCl2, 0.33 mM MgSO4, and 0.00001% methylene blue. Maintenance and breeding: WT AB strain Danio rerio at 26.5 °C. Embryos were collected in the morning of fertilization, raised until 46 hpf (hours post fertilization) at 28 °C in embryo medium prepared as described above, further selected and staged as previously described. After treating with NR-LUM@Azo-NCs for 24 h, embryos were selected for the further experiment, by transferring into medium containing 10 μM of copper sulfate to induce ROS conditions, for additional 3 h incubation. Zebrafish embryos incubated in a normal medium after treating with NR-LUM@Azo-NCs were used as the control group. Inverted fluorescent microscope was employed to observe the fluorescence distribution and intensity in zebrafish embryos.

Results and Discussion

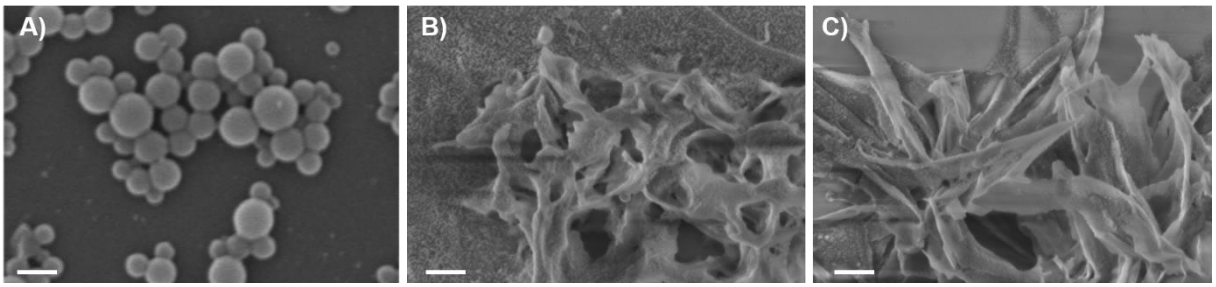

Figure S1. SEM images of DPI-LUM@Azo-NCs in the absence (A) and the presence of 3 μg/mL of HRP and 0.5 mM of H_2O_2 for (B) 0.5 h, and (C) 2 h. Scale bar 200 nm.

Under high ROS conditions with H_2O_2 (0.5 mM) in an aqueous solution, the surface of NCs folded during the first half hour, and gradually transformed into irregularity thin 2D film/sheets structure with increased aggregation.

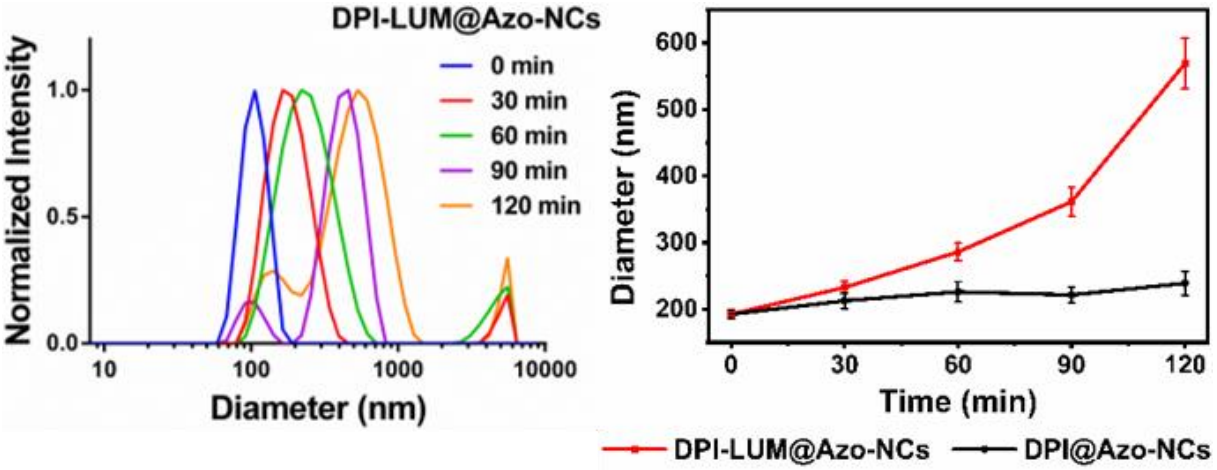

Figure S2. Time-evolved DLS analysis of DPI-LUM@Azo-NCs and DPI@Azo-NCs treated with 3 μg/mL of HRP and 0.5 mM of H_2O_2.

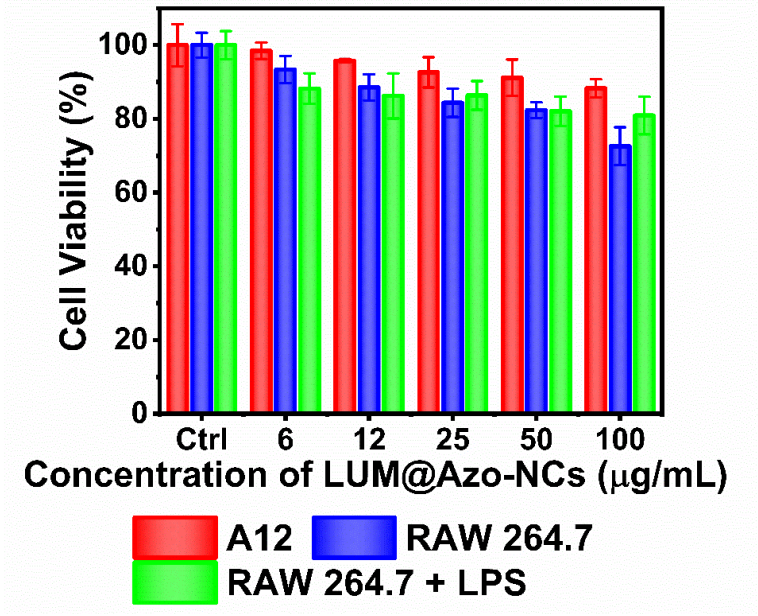

Figure S3. Cytotoxicity of LUM@Azo-NCs against A12, and RAW 264.7 (with or without LPS) cells after incubation for 36 h.

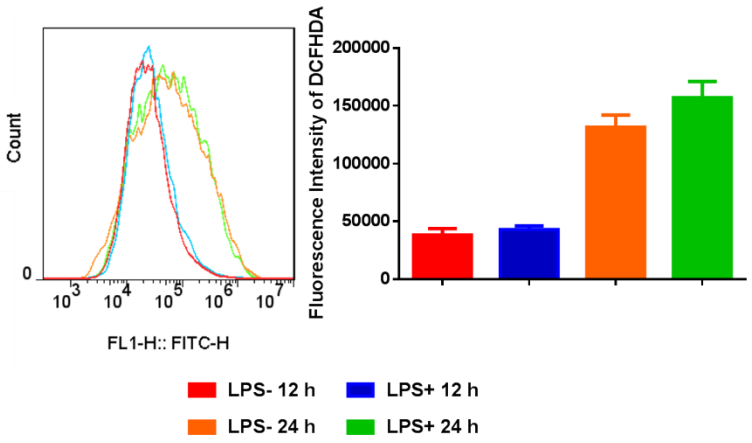

Figure S4. Flow cytometry analysis and the quantitative DCFHDA fluorescence intensity of the RAW 264.7 cells incubated with or without 100 ng/mL of LPS for 12 and 24 h, respectively. The cells were stained by DCFHDA for 2 h.

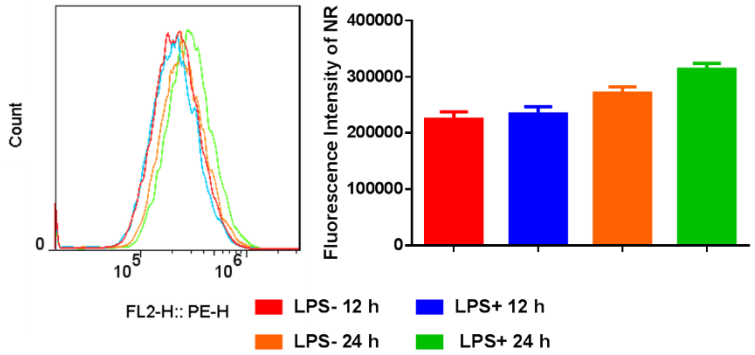

Figure S5. Flow cytometry analysis and quantitative NR fluorescence intensity of the RAW 264.7 cells incubated with NR-LUM@Azo-NCs for 12 h, and subsequently incubated with and without 100 ng/mL of LPS for 12 and 24 h, respectively.

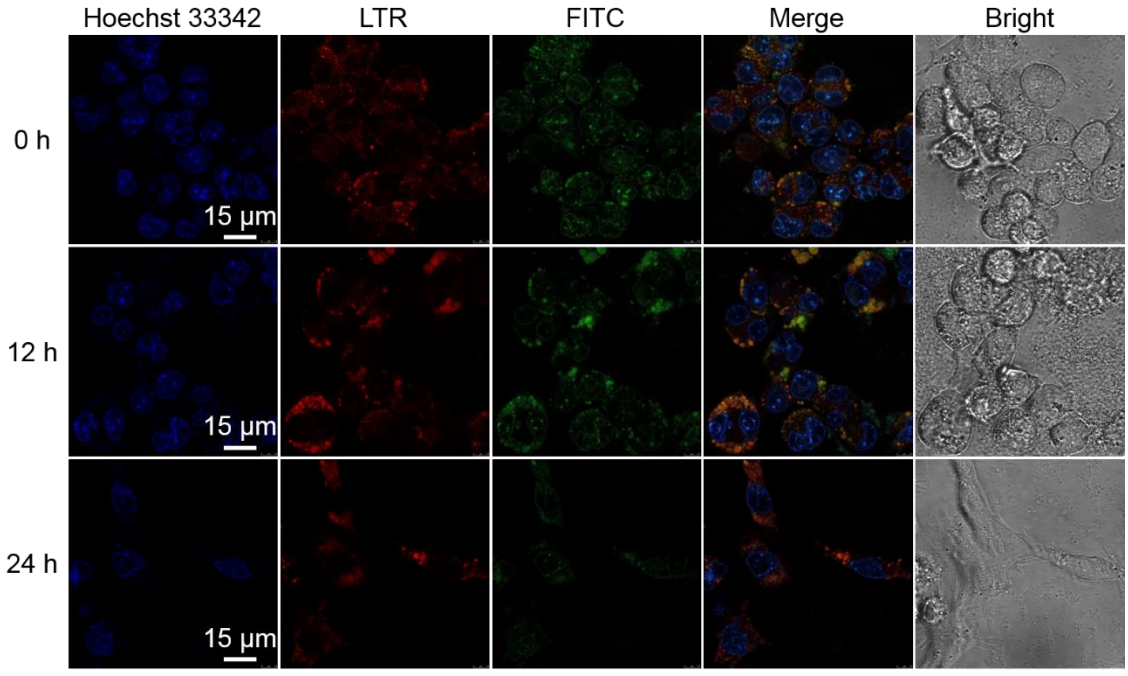

Figure S6. CLSM images of RAW 264.7 cells incubated with LUM@Azo-NCs@SP-FITC for 24 h, and subsequently incubated with 100 ng/mL of LPS for 0, 12 and 24 h. Hoechst 33342, ex/em=405/440-480 nm, LTR (Lyso-Tracker Red), ex/em=577/580-600 nm, SP-FITC, ex/em=488/505-545 nm.

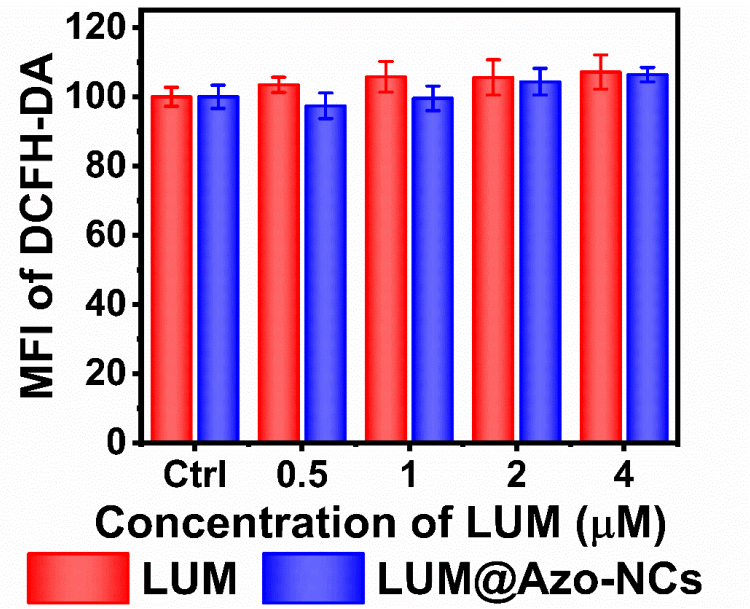

Figure S7. ROS generation of inflammatory RAW 264.7 cell line respectively treated with LUM and LUM@Azo-NCs for 24 h, and stained by DCHF-DA for 2 h.

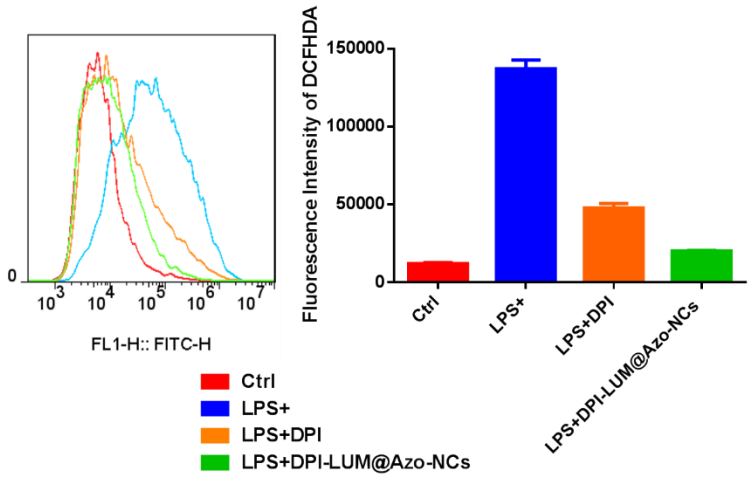

Figure S8. Flow cytometry analysis and the quantitative DCFHDA fluorescence intensity of the RAW 264.7 cells incubated with 100 ng/mL of LPS for 12 h, followed with incubation with DPI and DPI-LUM@Azo-NCs (including 100 nM DPI) for 24 h, respectively. The cells were stained by DCFHDA for 2 h.

References

[1] Jon, S. Y.; Selvapalam, N.; Oh, D. H.; Kang, J.-K.; Kim, S.-Y.; Jeon, Y. J.; Lee, J. W.; Kim, K., Facile synthesis of cucurbit [n] uril derivatives via direct functionalization: expanding utilization of cucurbit [n] uril. *J. Am. Chem. Soc.* **2003,** *125*, 10186-10187.