Electronic Supplementary Material (ESI) for Materials Chemistry Frontiers.

Supplemental Material

Lipid Droplets Formation and Dynamics: Tracking by Time-resolved Fluorescence Imaging

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1. Materials

All the chemicals were purchased from J&K. Commercially available reagents were used without further purification. DSPE-mPEG2000 was purchased from Shanghai Pharmaceutical Co., Ltd. Dulbecco's modified Eagle's medium (DMEM) and penicillin−streptomycin solution (100×) were purchased from Corning Incorporated. Fetal bovine serum (FBS) and BODIPY^{TM} 493/503 were purchased from Thermo Fisher Scientific. Calf serum was purchased from Gibco. 3-(4,5-Dimethylthiazol-2-yl)-5-(3-carboxymethoxy-phenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) was purchased from Promega Corporation. Insulin, indomethacin, Dexamethasone (Dex) and 3-Isobutyl-1-methylxanthine (IBMX) were purchased from Sigma-Aldrich. 3,3',5-Triiodo-L-thyronine (T3) was purchased from Cayman Chemical. Antifading mounting medium was purchased from Beijing Solarbio Science & Technology Co., Ltd.

2. Cell culture and viability assay

The human cervical cancer cell line (HeLa) and human hepatoma cell line (HepG 2) used in our experiments were purchased from Cell Resource Centre, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences & Peking Union Medical College. HeLa and HepG 2 cells were cultured in DMEM containing 10% FBS and 1% penicillin−streptomycin. All cell lines were maintained at a humidified incubator with 5% CO_2 at 37 ^{o}C. The cytotoxicity of **AI-Cz-M** was determined by MTS assay *in vitro*. The HeLa or HepG 2 cells were respectively seeded on each well of 96-well flat-bottom microtiter plates at a density of 1×10^4 cells/mL with 100 μL per well. After 24 h of culture, different concentrations of **AI-Cz-M** (0-50 μM) were added and further incubated for 24 h. After treatment, MTS solution (20 μL) was added to each well and continued to incubate for 3 hours. After 3 hours incubation at 37 ^{o}C, the absorbance of

sample and control wells at 490 nm was then measured by Tecan Spark™ 10M Multimode Microplate Reader. Cell viability was calculated according to the following formula: Cell viability (%) = $(A-A_0)/(A_s-A_0) \times 100$, where A is the absorbance of the experimental group, A_0 is the absorbance of the blank group (no cells), and A_s is the absorbance of the control group (no probe). Each of the experiment was repeated three times.

3. Cell treatment with oleic acid

HeLa or HepG 2 cells were grown overnight on an 8-well chamber containing sterile coverslips at the bottom. The cells were exposed to a series of doses of oleic acid (0–50 μM) for certain time (0–6 h) to induce lipid droplet formation. The oleic acid-treated cells were then incubated with **AI-Cz-M** (10 μM) for 2 hours.

4. Differentiation of 3T3-L1 preadipocytes into mature adipocytes

3T3-L1 cells were seeded on 30 mm glass bottom dishes at a density of $3x10^5$ cells per dish in DMEM medium with 10% calf serum. Upon reaching about 90% confluence, the medium was changed to DMEM High Glucose medium with 10% FBS (day -2). Two days post confluency (day 0), cells were cultured for 2 days in differentiation medium including insulin (50 nM), T3 (100 nM), indometacin (0.125 mM), Dex (2 μg/ml) and IBMX (0.5 mM). Then, the cells were cultured in regular medium containing insulin (50 nM) and T3 (1 nM) for 2 (day 2), 4 (day 4) and 6 (day 6) days, respectively. After induction, the cells were incubated with **AI-Cz-M** (10 μM) in DEME medium (500 μL/dish). After incubation at 37 ^oC for 2 hours, the cells were washed three times with PBS buffer and finally were imaged under confocal microscope.

5. Cellular uptake analysis of micelles by flow cytometry

The influence of temperature on cellular uptake of **AI-Cz-M** was quantitatively detected by a flow cytometry system (FCS, ZE5 Cell Analyzer, Bio-rad, USA). HeLa cells were seeded in 35 mm petri dishes with a cover slip at a density of approximately 3×10^5 cells per dish and incubated for 24 hours at 37 °C under 5% CO_2. The culture medium was then removed, and the cells were washed twice with PBS, followed by incubation with 50 μM oleic acid in DMEM medium at 37 °C. After 5 h incubation, the medium was replaced by 10 μM **AI-Cz-M** in serum-free medium and incubated at 37 ^oC and 4 ^oC for 0.5 hour, 1 hour, 2 hours and 4 hours, respectively. HeLa cells incubated with serum-free medium were used as control. After that, the medium was removed, and the cells were rinsed with cold PBS for three times to abort endocytosis, trypsinized and harvested with 0.4 mL of 0.25% (w/v) trypsin solution, collected by centrifugation followed by washing twice with PBS, and then resuspended in 500 μL of precold PBS followed by filtration through a nylon mesh. Finally, the intracellular fluorescence intensity was determined by FCS at 405 nm excitation wavelength and 525/50 nm emission wavelength. For each sample, 10000 events were collected and the data were shown as the mean value.

Table S1. Fluorescence lifetime compositions of delayed components of **AI-Cz-M** in normal saline solution without deoxygenating. Excited at 377 nm and monitored at 540 nm.

	τ_1[a] (μs)	n_1%[b]	τ_2[a] (μs)	n_2%[b]	τ[c] (μs)
AI-Cz-M	44.65	13.92	305.3	86.08	299.28

[a]Obtained from the double-exponential fitting of transient decay curves on a 2 ms scale; [b]the contribution of each component to average lifetime; [c]the average fluorescence lifetime of the delayed component. Double-exponential fitting: $\tau = (n_1\tau_1^{\ 2} + n_2\tau_2^{\ 2})/(n_1\tau_1 + n_2\tau_2)$.

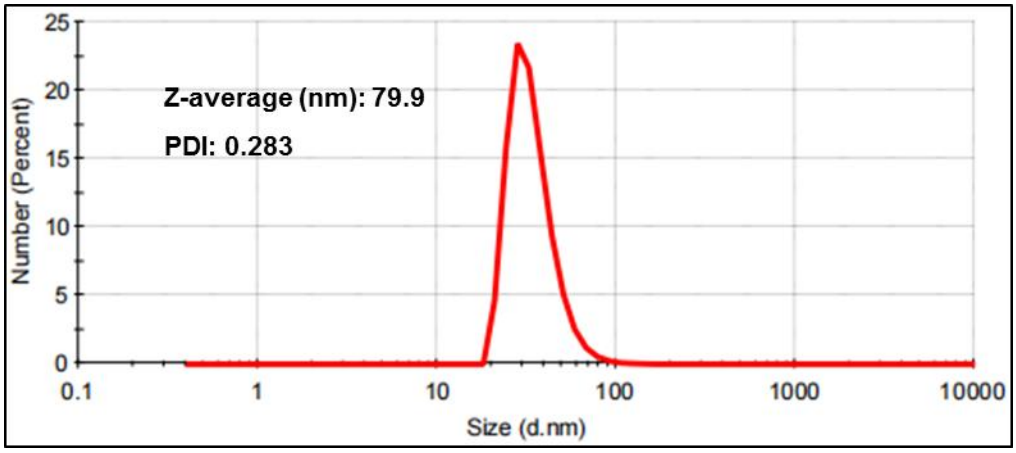

Fig. S1 Representative size distributions of **AI-Cz-M** measured by DLS after being stored in aqueous solution for almost 10 months.

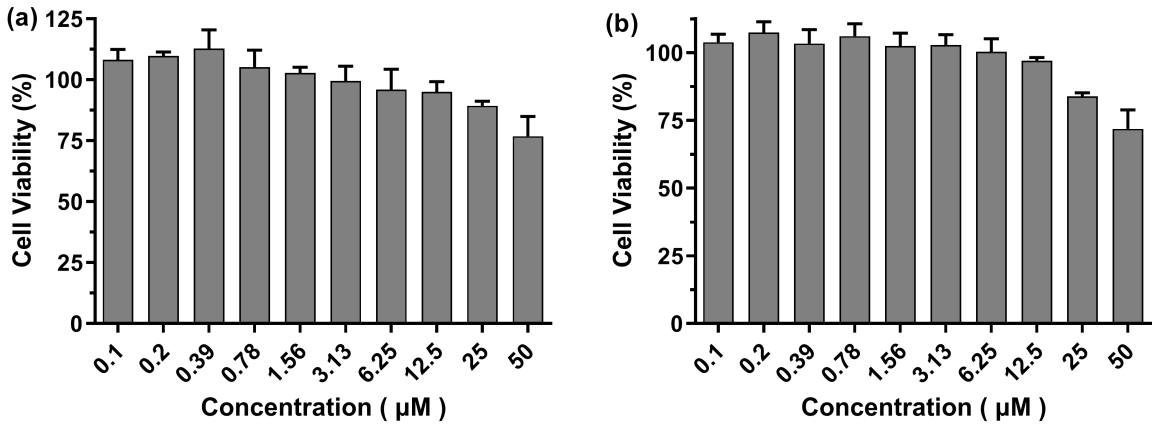

Fig. S2 Cell viability of **AI-Cz-M** in HeLa cells and HepG 2 cells at various concentrations after 24 hours of incubation. Mean value and standard deviations were obtained from three independent experiments.

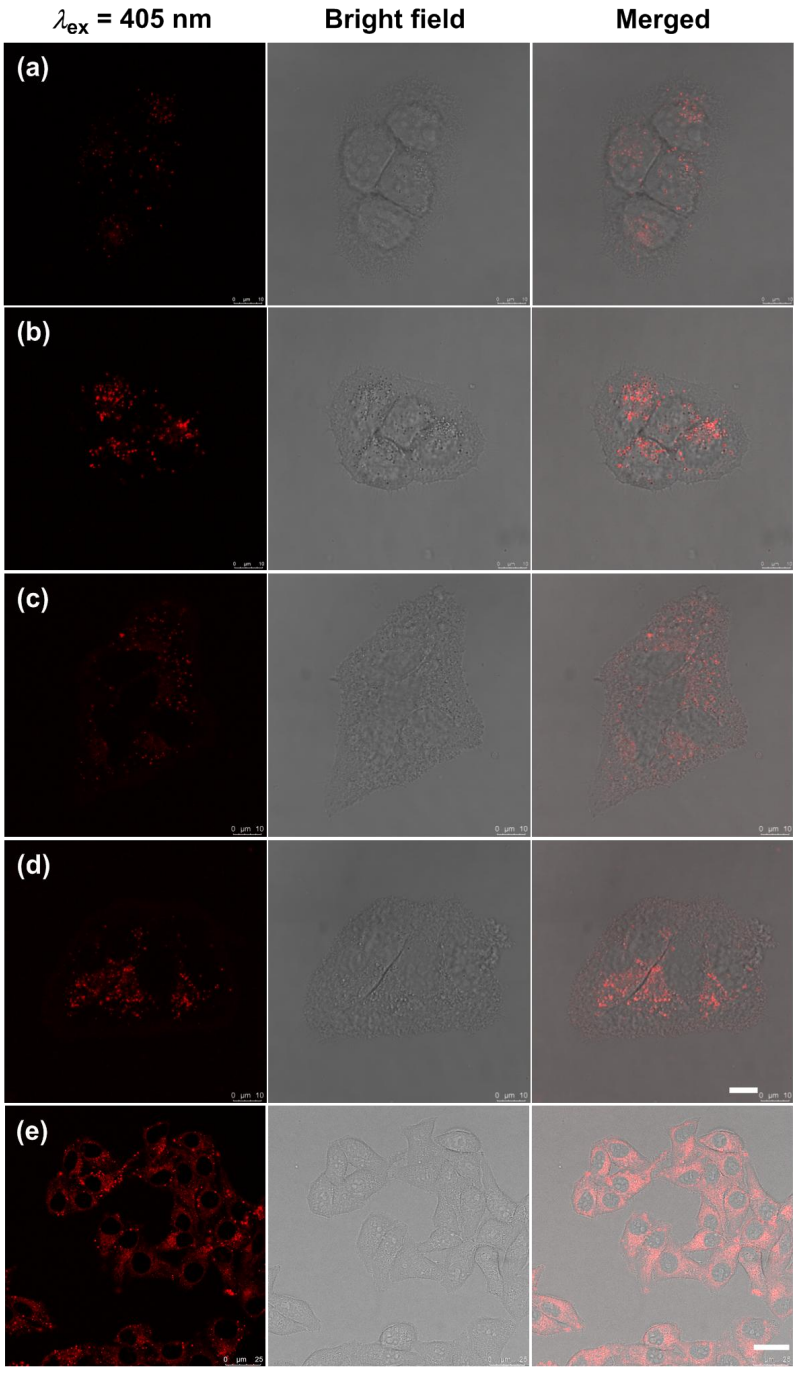

Fig. S3 Confocal images of HeLa cells (a, b) and HepG 2 cells (c, d) stained with **AI-Cz-M (**10 μM) in the absence (a, c) or presence (b, d) of 50 μM oleic acid pre-treatment for 6 h, respectively. (e) Confocal images of HepG 2 cells stained with **AI-Cz** (10 μM) without oleic acid pre-treatment. Probe signal (red): λ_{ex} = 405 nm and λ_{em} = 450-700 nm. (a-d) Scale bar = 10 μm; (e) Scale bar = 25 μm.

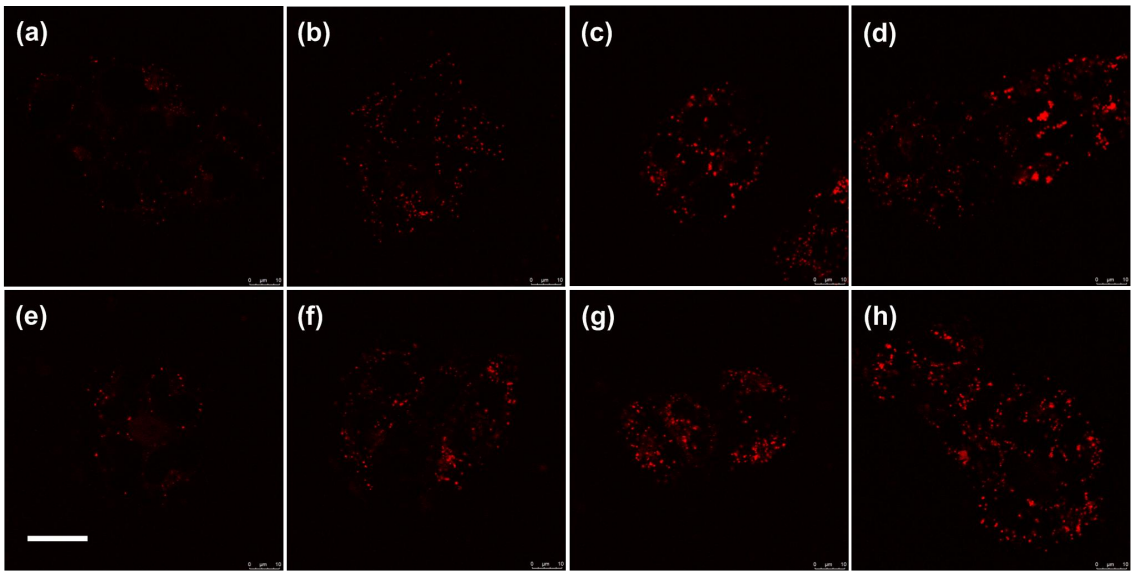

Fig. S4 (a-d) Confocal images of HeLa cells incubated with oleic acid (50 μM) for (a) 0 h, (b) 2 h, (c) 4 h, (d) 6 h, followed by staining with **AI-Cz-M** (10 μM). (e–h) Confocal images of HeLa cells incubated with (e) 0 μM, (f) 12.5 μM, (g) 25 μM and (h) 50 μM oleic acid for 6 h, followed by staining with **AI-Cz-M** (10 μM). **AI-Cz-M** signal: λ_{ex} = 405 nm, λ_{em} = 450-700 nm. Scale bar = 20 μm.

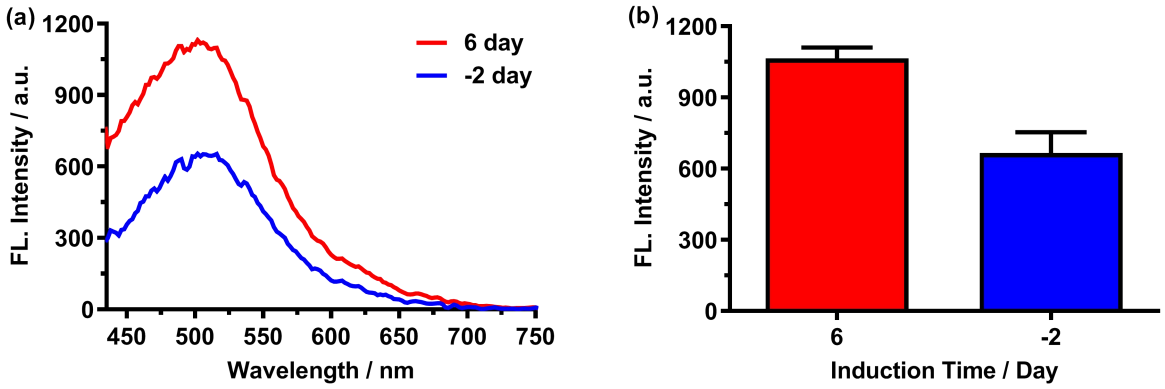

Fig. S5 Fluorescence intensity of 3T3-L1 adipocytes cells treated with **AI-Cz-M** (10 μM) for 2 h before induction (day -2) and 6 days after induction (day 6). Error bars show SEM. λ_{ex} = 390 nm.

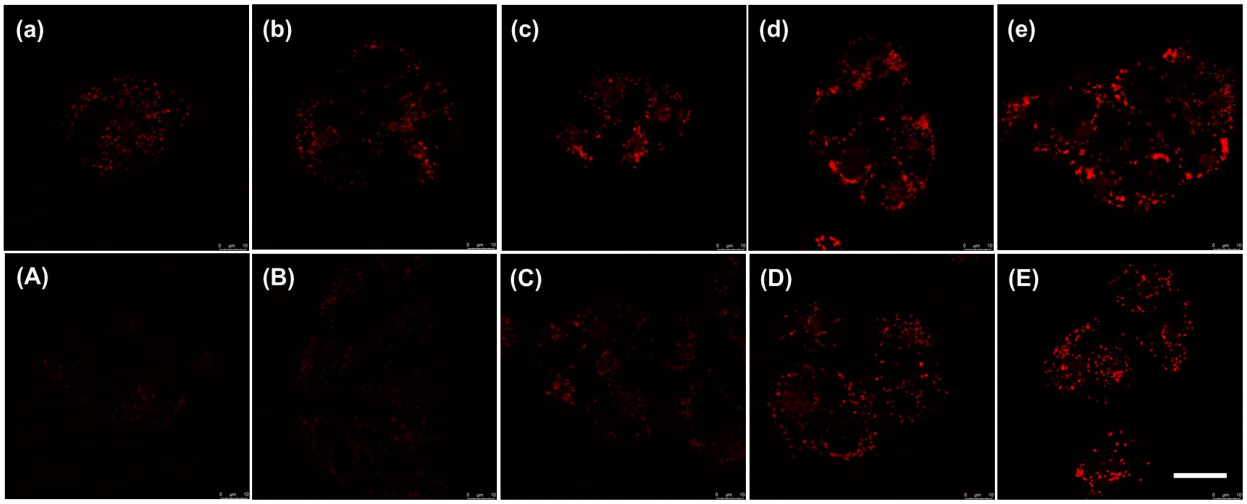

Fig. S6 Confocal images of the uptake process of **AI-Cz-M** in HeLa cells after treatment with (a) 5 μM, (b) 10 μM, (c) 15 μM, (d) 20 μM and (e) 30 μM **AI-Cz-M** for 2 h. (A-E) Confocal images of HeLa cells stained with 10 μM **AI-Cz-M** for (A) 10 min, (B) 30 min, (C) 1 h, (D) 2 h and (E) 4 h, respectively. **AI-Cz-M** signal: λ_{ex} = 405 nm, λ_{em} = 450-700 nm. Scale bar = 20 μm.

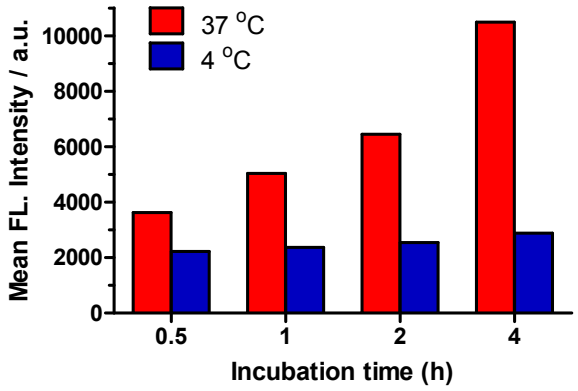

Fig. S7 Cellular uptake of 10 μM **AI-Cz-M** in HeLa cells at different temperatures (37 °C and 4 °C) with various incubation times. The intracellular fluorescence intensity was determined by FCS at 405 nm excitation wavelength and 525/50 nm emission wavelength. For each sample, 10000 events were collected and the data were shown as the mean value.

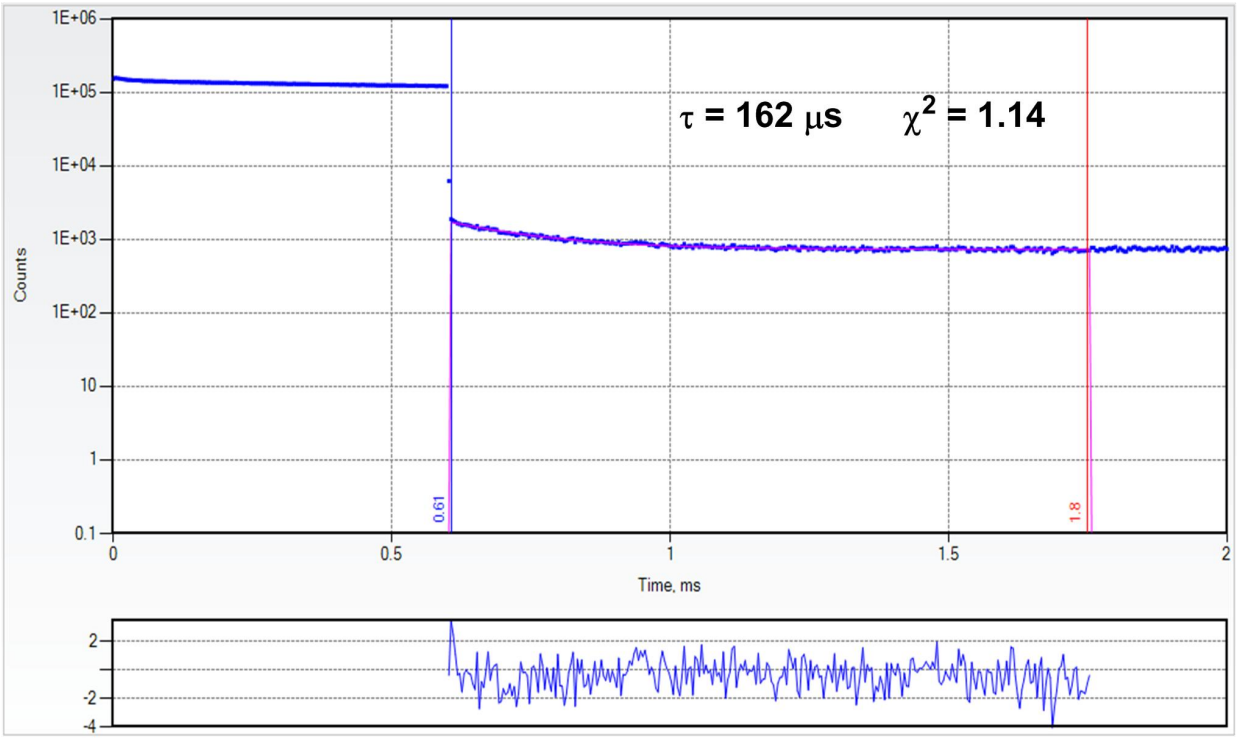

Fig. S8 Corresponding fluorescence lifetime decay curve with reliable one exponential fitting of **AI-Cz-M** in HeLa cells.