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Letter

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New Fluoranthene FLUN-550 as a Fluorescent Probe for Selective Staining and Quantification of Intracellular Lipid Droplets

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Supporting Information

ABSTRACT: A new class of live cell permeant, nontoxic fluoranthene-based fluorescent probe (FLUN-550) having a high Stokes shift in aqueous medium has been discovered. It showed selective staining of lipid droplets (LDs, dynamic cytoplasmic organelles) at a low concentration without background noise in in vitro live cell imaging of 3T3-L1 preadipocytes, J774 macrophages, MCF7 breast cancer cells, and single-celled, parasitic protozoa *Leishmania donovani* promastigotes and in vivo nonparasitic soil nematode *C. elegans*.

Fluorescence imaging tools have played an indispensable role in unequivocally unraveling the mysteries of many problems in lipid cell biology. Therefore, the development of new fluorescent probes for cell-based screening platforms to visualize, quantify, and/or study the molecular dynamics of lipid droplets (LDs) is highly rewarding for technological advancements in the area of molecular and cell biology.[1]

The most widely used fluorescent lipid dyes, which stain intracellular lipid components such as LDs, are Nile Red,[2] BODIPY[3] (Figure 1), and recent dyes like LD-540,[4] LipidGreen,[5] MDH,[6] and Seoul-Fluor.[7] Undoubtedly, these LD markers have significantly contributed to the elucidation of the molecular mechanisms associated with lipid biology; however, the commonly used probes (Nile Red, BODIPY) have small Stokes shifts and thus induce nonradiative energy transfers causing fluorescence quenching of the excited chromophore and interference from scattered light ultimately leading to weak fluorescence and background artifacts.[8] Researchers have recently addressed these issues and developed BODIPY derivatives with an as yet largest Stokes shift of about 185 nm.[9] The high Stokes shifts fluorophores have key advantages in multimodal cell imaging. Herein we report a new[10] cell-permeable solvatochromic fluoranthene-based FLUN-550 fluorescent probe with high Stokes shift (220 nm) in water and demonstrate its potential application in selective staining and quantification of intracellular LDs in in vitro live 3T3-L1 preadipocytes, *Leishmania donovani* promastigotes, and in in vivo soil nematode *C. elegans*.

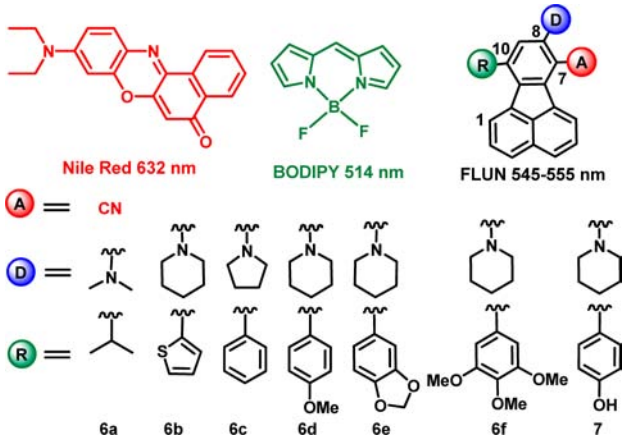

Figure 1. Structures of known lipid droplets markers and synthesized new fluoranthenes (FLUNs, **6a−f**, **7**); A-acceptor, D-donor, R-alkyl, aromatic group.

Our research group is involved in developing new small molecule organic fluorescent dyes for their potential applications in applied sciences (as electroluminescent materials).[11] We have shown the importance of positioning the electron donor−acceptor and chromophoric moieties onto the molecular frameworks for modulating the photophysical properties of fluorenes,[12] pyrenylarenes,[13] benzo[*f*]quinolines,[14] and fluoranthenes[15] leading to efficient multicolor light emitting materials for organic electronics.

Owing to the interesting photophysical and optical properties of highly fluorescent organic dyes, particularly fluoranthene (FLUN) derivatives, we tuned them by incorporating donor and acceptor functionalities. Several new derivatives of FLUN with hydroxy and methoxy groups (Figure 1) were synthesized in high yields and purities as fluorescent probes for cell-imaging applications (see Scheme S1 in the Supporting Information).

The photophysical properties of all of the synthesized fluoranthenes (FLUNs) **6a−f** and **7** in water containing 0.2%

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DMSO showed absorption maxima in the range of 327−340 nm with a low intensity band (n→π^*) in the range of 428−458 nm and photoluminescence (PL) maxima in the range of 545−555 nm (see Table S1 and Figures S1−S7 in the Supporting Information). These FLUNs showed high Stokes shifts in aqueous medium as shown in Figure 2 for compound 7

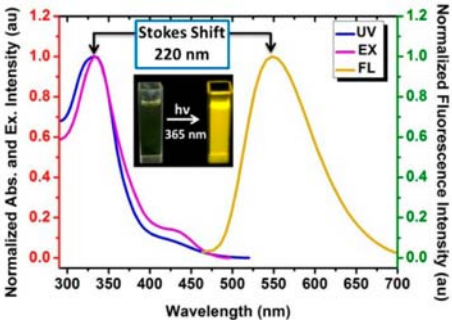

Figure 2. Absorption, excitation, and emission spectra of FLUN-550 dye in 0.2% DMSO in water (~10^{-5} M).

(FLUN-550, $\lambda_{max, PL}$ 550 nm; Stokes shift 220 nm) with a quantum yield of ~4% in water and 35% in DMSO. To study the effect of the polarity of the solvent on the excited state of the compounds, we examined the absorption and PL spectra of FLUNs using solvents of varying polarity. Interestingly large solvatochromic shifts were observed in the PL spectra for FLUN derivatives (Figure S8 in the Supporting Information). The positive solvatochromism indicated that the donor−acceptor fluoranthene FLUN-550 exhibited a strongly stabilized excited state compared to the ground state by the surrounding solvent molecules (see Table S2 in the Supporting Information).

In order to examine the cellular permeability/uptake, fluorescent FLUN dyes **6a−f** and **7** were incubated in populations of J774 mouse macrophages and MCF7 cells under growth conditions (see the Supporting Information). Flow cytometry (FACS) analysis of J774 and MCF7 cells revealed that the hydroxy group at the benzene periphery at position 10 (FLUN-550) displayed nice cellular uptake as compared to other methoxy (**6d**), methylenedioxyphenyl (**6e**), or other substituents. Cytometric results for dye-untreated (with blank medium) J774 and MCF7 cells showed 121 and 255 au intensity at 405 nm excitation wavelength, respectively, whereas FLUN-550-treated J774 cells and MCF7cells showed intensities shifting from 121 au (control) to 6804 au and from 255 au to 8818 au, respectively. Higher emission intensities confirmed the high cell permeability of FLUN-550 (Figure 3).

On the basis of the cellular uptake studies on fluoranthene derivatives (**6a−f** and **7**), FLUN-550 was chosen for selectivity

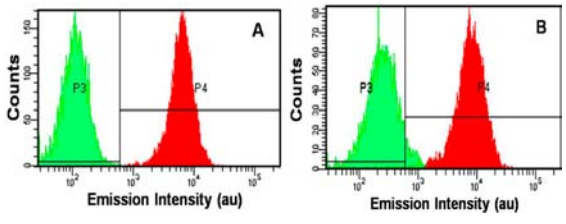

Figure 3. Flow cytometry analysis (shift in emission intensity) of FLUN-550 (7) at 50 nM after 2 h incubation in (A) J774 cells and (B) MCF7 cells. P3 corresponds to control cells, whereas P4 corresponds to cells treated with FLUN-550.

studies in live J774 macrophages cells and MCF7 breast cancer cells using confocal laser scanning microscopy. After 2 h incubation with dye FLUN-550 (at 50 nM), we observed good intracellular fluorescence with a high signal-to-noise ratio, which was attributed to the FLUN-550 (Figure 4). These results indicated that the dye is capable of entering into the cytoplasm but not in the nucleus.

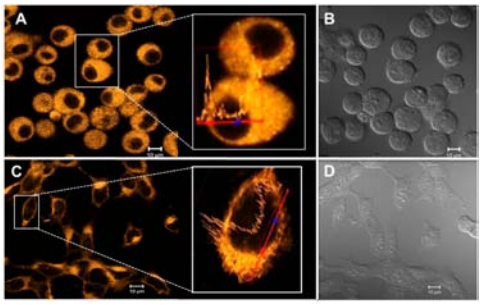

Figure 4. (A) Confocal microscopy images of live J774 cells with intensity line plot; (B) DIC image; (C) confocal microscopy images of live MCF7 cells with intensity line plot; (D) DIC image.

Since mammalian J774 macrophages cells store excess unesterified cholesterol in cytoplasmic lipid droplets (LDs),[16] we envisioned the fluorescent staining in cytoplasm by the fluorescent dye FLUN-550 may be due to the staining of large number of small LDs present in the cytoplasm as described by Neef and Schultz.[1a] In order to examine the selective staining of FLUN-550 for LDs, we further investigated the staining of the dye FLUN-550 in 3T3-L1 preadipocyte cells, which are known to house LDs in large numbers. Interestingly, confocal results revealed that FLUN-550 selectively stained the lipid droplets (LDs) of preadipocytes at the optimized concentration of 100 nM (Figure 5). Quantification of lipid droplets was achieved using image J software, which accounted for 192 lipid droplets.

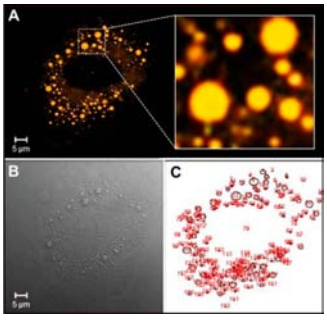

Figure 5. 3T3-L1 preadipocytes stained with 100 nM of FLUN-550 for 30 min at 37 °C/5% CO_2 on confocal dishes: (A) confocal fluorescence image; (B) phase image; (C) quantification of lipid droplets using image J software. Total LDs quantified is 192.

To confirm these observations, dual-staining experiments were performed with Nile Red (a known lipid droplets marker) and Hoechst-33342 (nucleus staining dye) in 3T3-L1 preadipocytes, which revealed specific localization in distinct cytoplasmic lipid droplets present in these cells (Figure S9 and movie-1 in the Supporting Information) and clearly showed colocalization of the Nile Red and FLUN-550 within LDs (see Figure S10 in the Supporting Information). Cell viability experiments revealed that FLUN-550 showed no cytotoxicity in 3T3-L1 adipocytes up to a concentration of 1 μM, where

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93.5% cells were viable (see Figure S11 in the Supporting Information).

Considering the importance of lipid metabolism events in live *Leishmania donovani* parasites, we further explored the potential of FLUN-550 for selective staining of lipids in live *L. donovani* parasites, the causative protozoan for the disease visceral leishmaniasis (KALA-AZAR).[17,18] Microscopic observations of *L. donovani* stained with FLUN-550 revealed localized fluorescence within specific spherical compartments of the cells with 99% cell viability (see Figure 6 and Figure S12 in the Supporting Information).

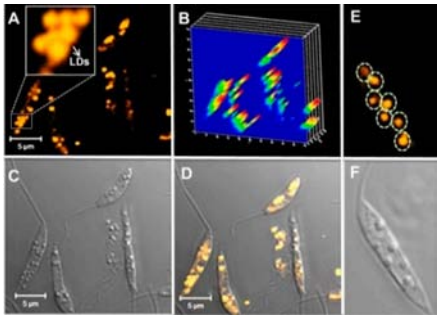

Figure 6. Confocal image of *L. donovani* promastigotes stained with 100 nM FLUN-550: (A) fluorescence image; (B) pseudo-3D fluorescence intensity plot of lipid droplets; (C) phase image; (D) merge of image A and C (bar indicates 5 μm); (E) quantification of lipid droplets (LDs encircled) by fluorescence staining with FLUN-550; (F) phase image.

Furthermore, dual staining of *L. donovani* promastigotes with Nile Red and FLUN-550 confirmed the specific localization in distinct LDs with no labeling in nucleus and other organelles present in these cells (see Figure S13 in the Supporting Information). Due to the strong and specific labeling of LDs with FLUN-550, the quantity and size of the stained lipid droplets served as a biotool for investigating lipid metabolism (Figure 6E,F).

We further endeavored to study in vivo lipid specificity of the fluorescent dye in nonparasitic soil nematode *C. elegans*, which is of relevance in studying lipid biology. We observed that treatment of these nematodes with different concentrations of FLUN-550 dye led to a concentration-dependent staining pattern with 1 μM concentration exhibiting the most robust fluorescence staining pattern (Figure 7). Staining was observed throughout the worm body with specific lipid droplets fluorescing in the head region around the pharynx, alongside the gut region, and leading all the way to the tail region of the worms. The staining was more intense toward the midbody, which usually possesses high lipid content in gravid well-fed adults (see movie-2 in the Supporting Information). The hallmark of the staining with FLUN-550 dye was its specificity to the body lipids within the worms. There was no nonspecific fluorescence within the nematode gut as is seen in case of FLUN-550 (black arrowheads, Figure 7D). The colocalization studies, wherein FLUN-550 and Nile Red were supplemented together, showed complete overlap of the lipid droplets stained by either dye, with the FLUN-550 dye exhibiting rather sturdier fluorescence (spots for comparison marked with white arrowheads in Figure 7A,B). Comparison of emission maxima of commercially available dyes BODIPY (514 nm) and Nile Red (632 nm) with FLUN-550 (550 nm) showed a clearly distinct region with the advantage of having remarkably high Stokes shift, which is an important parameter in cell imaging applications.

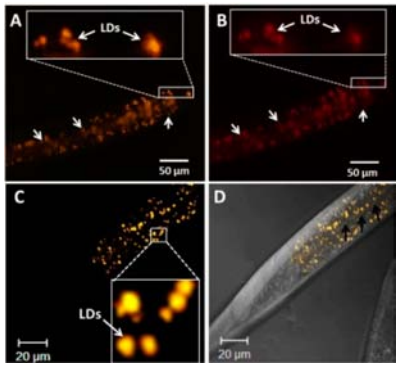

Figure 7. (A) Fluorescence microscopic image of *C. elegans* stained with 1 μM FLUN-550. (B) Fluorescence microscopic image of *C. elegans* stained with 1 μM Nile Red. (C) Confocal fluorescence image of *C. elegans* stained with 1 μM FLUN-550. (D) Merged DIC image.

In conclusion, we have discovered a new class of live cell permeant, fluorescent probe FLUN-550 for strong and selective staining of intracellular lipid droplets in in vitro live 3T3-L1 preadipocytes, J774 macrophages, MCF7 breast cancer cells, parasitic protozoa *L. donovani* promastigotes, and in vivo nonparasitic soil nematode *C. elegans*. The live cell imaging studies clearly demonstrated that FLUN-550 is a specific and nontoxic lipid molecular probe for quantification of lipid droplets with high brightness at a low concentration without background noise. FLUN-550 exhibited clearly distinct PL from the commercially available BODIPY and Nile Red dyes, and it may be used as a fluorescent probe alone or in combination with other fluorophores for multicolor imaging applications.

■ ASSOCIATED CONTENT

Supporting Information

Synthesis, ^{1}H and ^{13}C NMR, and UV-FL spectra of compounds **6a−f** and **7**. Cytotoxicity data, dual staining and colocalization in 3T3-L1 preadipocytes and *L. donovani*, movie-1 (LDs staining in 3T3-L1 preadipocytes), and movie-2 (LDs staining in *C. elegans*). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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DEDICATION

Dedicated to Prof. T. K. Chakraborty on the occasion of his voluntary retirement from CSIR-CDRI, Lucknow.

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