Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications

Supplementary Information

Confocal Microscopy of Cytoplasmic Lipid Droplets in a Live Cancer Cell: Number, Polarity, Diffusion and Solvation Dynamics

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Experimental Section

1. Materials

Dimethylsulfoxide (DMSO) for cell culture, Dulbecco's Modified Eagle Medium (DMEM), coumarin 153 (C153, Exciton, Scheme 1A) and 4′, 6-diamidino-2-phenylindole dihydrochloride (DAPI, Scheme1B) were purchased from Sigma Aldrich. Fetal bovine serum was purchased from Invitrogen. All the materials were used without further purification. Human lung cancer (A549) cell line were purchased from National Centre for Cell Science, Pune, India and cultured in our laboratory. Lung fibroblast cell was received as a gift from Dr. D. Sinha (IACS, Kolkata) and cultured in our laboratory.

2. Methods

2.1 Cell Preparation: Human lung cancer (A549) cells and non-cancer lung fibroblast (WI38) cells were grown in phenol red free DMEM with 10% fetal bovine serum, 1% Pen Strep Glutamine (from Gibco) in an atmosphere of 5% (v/v) CO_2 enriched air at 37 °C. A stock solution of C153 in biocompatible DMSO and DAPI in water (500 nM) were prepared. Cells were seeded at a density of 5000 cells per petri dish in a culture petri dish (BD BioCoat) for 18-24 hours before the dyes treatment. For proper staining of the cells, 200 μL of 500 nM dye solutions was added to the culture dish and incubated (half an hour for DAPI and 4 hours for C153) separately. After incubation the cells were washed 3-4 times with phosphate buffered

saline (PBS) and 200 μL fresh media was added to the petri dish. The petri dish, containing properly stained cells was used for imaging and recording fluorescence decays and emission spectra under the microscope. For FCS studies, 200 μL of the 50 nM dye solutions were added to the petri dish. All microscopic measurements were carried out at 20 °C.

2.2 Experimental Set-up for Confocal Microscopy

The confocal microscope (Olympus IX-71) and TCSPC setup (Pico Quant, Micro-Time 200) are described in our previous publications.[1] We used a water immersion objective of numerical aperture, NA=1.2. For one photon excitation (1PE), we used a picosecond diode with λ_{ex} = 405 nm. For two-photon excitation (2PE) experiment, a mode-locked Ti-sapphire laser of stable repetition rate ~ 80 MHz (Tsunami, Spectra Physics) was used. Since there is difficulty of recording emission decay at ~420 nm for DAPI because of scattered light, we have used two-photon excitation for DAPI. MPD detectors (Micro Photon Device, PDM series) were used for spectroscopic measurements. For recording the emission spectra under the confocal microscope, we used an EMCCD attached to a spectrograph (ANDOR Technology).

In order to record fluorescence decays using the confocal microscope, the parallel ($I_{\parallel}$, and perpendicular ($I_{\perp}$) components of fluorescence intensity were recorded by two MPD detectors and the decay at magic angle were obtained as follows,

$$I_{magic}(t) = I_{\parallel}(t)\cos^2(54.75^\circ) + GI_{\perp}(t)\sin^2(54.75^\circ)$$
$$= (1/3)I_{\parallel}(t) + (2/3)GI_{\perp}(t) \quad (1)$$

The instrument response function (IRF) is recorded using the back-scattered laser light from a bare slide (405 nm light for 1PE and 810 nm light for 2PE). The FWHM of the IRF for excitation at 810 nm (femtosecond laser) is found to be ~40 ps while for the excitation at 405 nm is ~90 ps. The fluorescence decays are deconvoluted by DAS6 v6.3 software. In order to avoid damage of cell, we have applied very low laser power of ~ 50 nW during recording of the fluorescence decays inside the live cell. The G-factor is determined by tail matching of the $I_{\parallel}$ and $I_{\perp}$ components of fluorescence and is found to be 1.8.

2.3 Analysis of Solvation Dynamics and FCS Data

Experimentally, solvation dynamics is followed by time-dependent red shift of the emission maximum of a polar solute to lower energy (Stokes shift) with increase in time. As a result of solvation dynamics, the fluorescence decay at the blue end (short wavelength, unsolvated species) exhibits a decay. At long emission wavelength (red end), the transient for the solvated

species exhibits a distinct rise preceding the decay. Thus for probe undergoing solvation dynamics the fluorescence decays are wavelength dependent. Wavelength dependent decays of DAPI inside the nucleus of a live cancer cell are shown in figure S1.

The time resolved emission spectra of C153 and DAPI in various regions of a lung cancer cell (A549) as well as non-cancer cell (WI38) (TRES, figure S2) were constructed from the steady state emission spectra and the fluorescence transients following by Maroncelli and Fleming.[2-3] The solvation dynamics is described by the decay of the solvent correlation function C(t), defined as,

$$C(t) = \frac{\nu(t) - \nu(\infty)}{\nu(0) - \nu(\infty)} \qquad (2)$$

where, $\nu(0)$, $\nu(t)$ and $\nu(\infty)$ are the emission maxima (frequencies) at time 0, t and ∞, respectively. The solvent correlation functions C(t) were fitted to a single-exponential or double-exponential decay as follows,

$$C(t) = \sum_{i=1}^{2} a_i e^{-\frac{t}{\tau_i}} \qquad (3)$$

For the analysis of FCS data, the auto-correlation function $G(\tau)$, were fitted to a 3D diffusion model[4] having a triplet contribution,

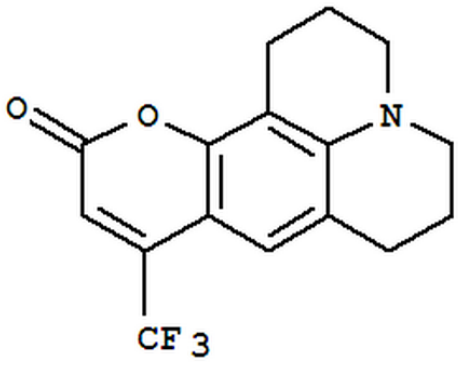
$$G(\tau) = \frac{1 - T + T\exp(-\tau/\tau_{tr})}{N(1-T)} \left(1 + \tau/\tau_D\right)^{-1} \left(1 + \tau/\tau_D S^2\right)^{-1/2} \qquad (4)$$

In the above equation, τ denotes the lag time, τ_D is the diffusion time of the dye molecule, N is the average number of molecules in the excitation volume, T represents the fraction of the dye molecules in the triplet state and τ_{tr} being the lifetime of the dye molecules in its triplet state. The structure parameter (*S*) of the excitation volume was calibrated using a sample (R6G in water) of known diffusion coefficient ($D_t \sim 426\ \mu m^2 s^{-1}$).[5] The estimated value of the confocal volume is found to be ~ 0.9 fL with a transverse radius (ω_{xy}) ~ 320 nm.

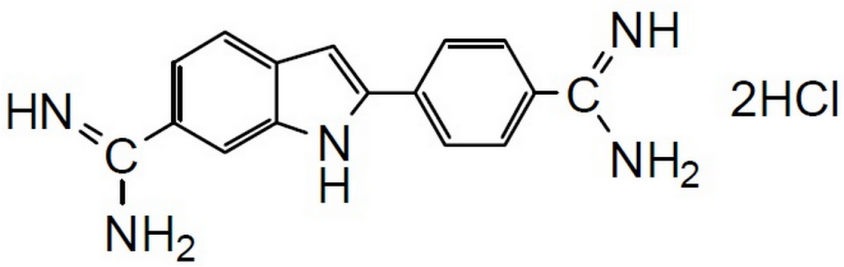
Diffusion coefficient (D_t) was calculated from the following equation,[6]

$$D_t = \frac{\omega_{xy}^2}{4\tau_D} \qquad (5)$$

Scheme 1



(A)



(B)

Scheme 1: Molecular structure of (A) Coumarin 153 (C153) and (B) DAPI.

Figure S1

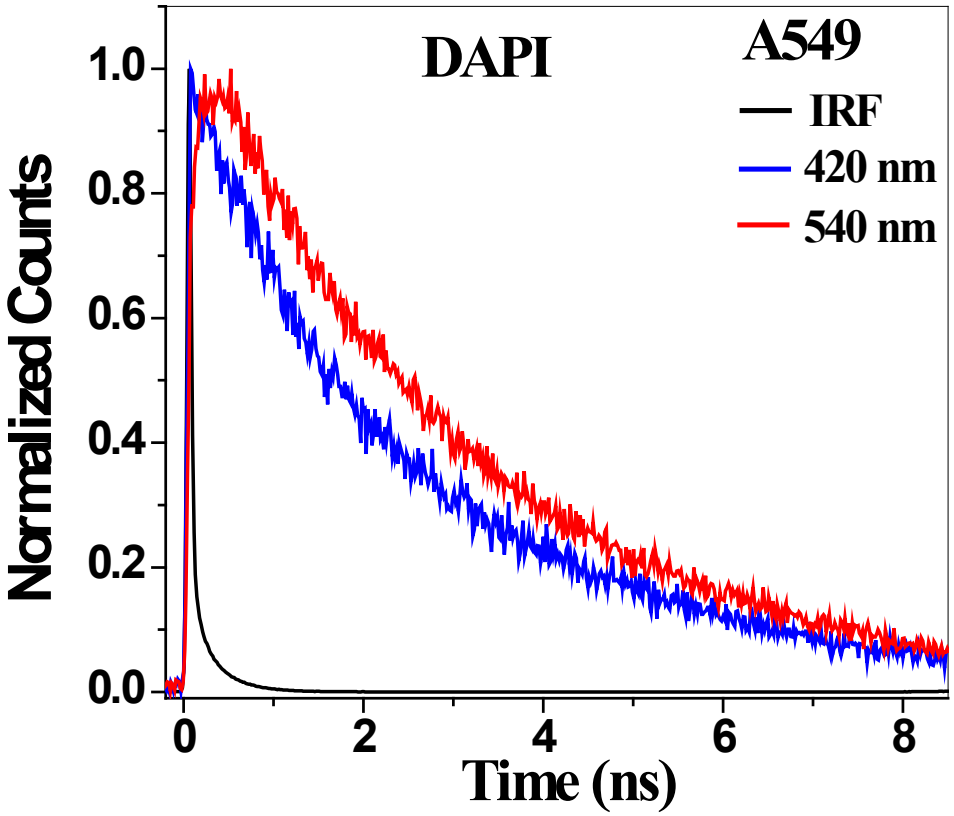


Figure S1. Picosecond decays of DAPI in nucleus of A549 cell (λ_{ex} = 810 nm, under 2PE excitation) at λ_{em} = 420 nm (blue) and at λ_{em} = 540 nm (red).

Figure S2

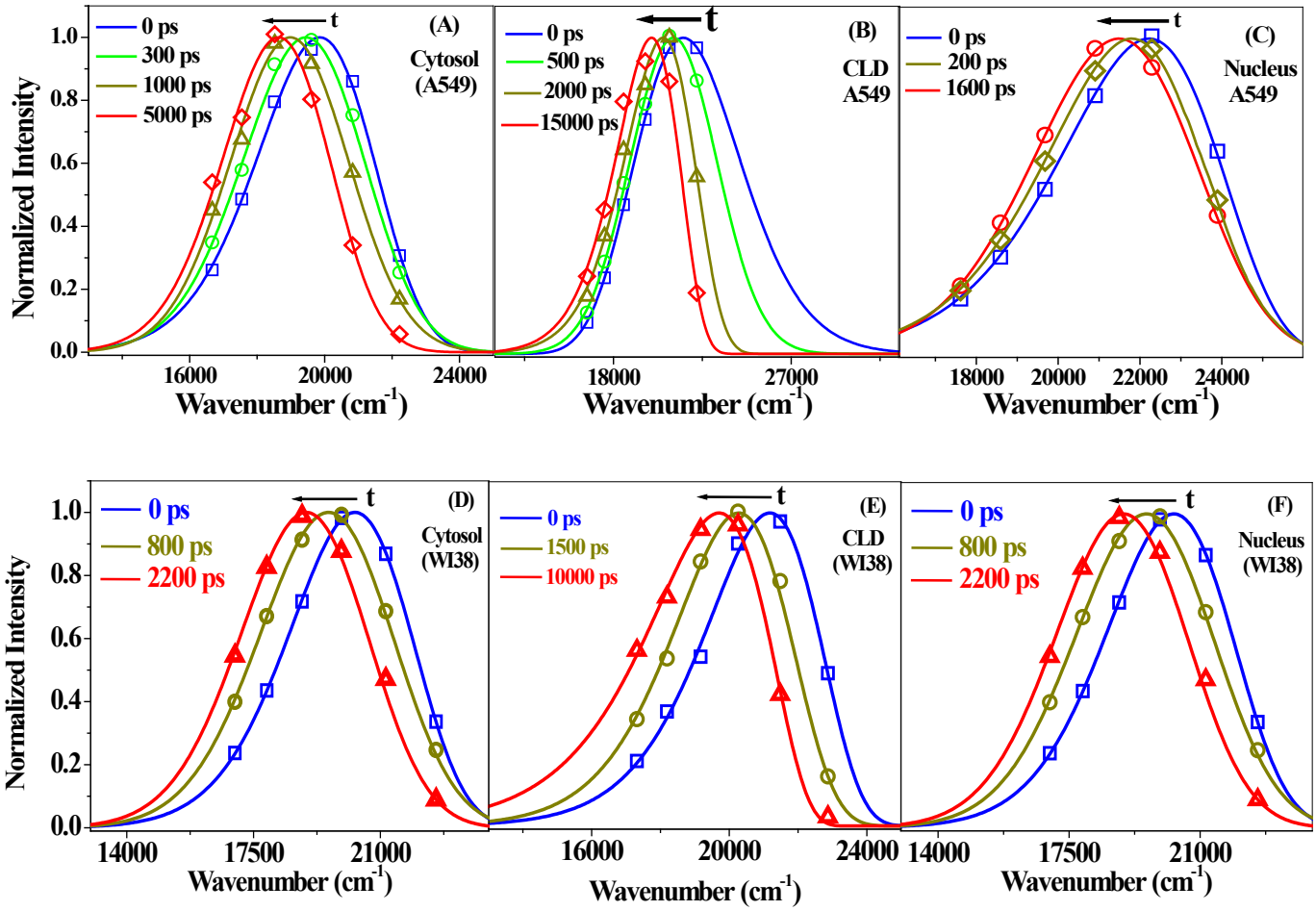


Figure S2. Time-resolved emission spectra (TRES) of C153 in cytosol (S2A and S2D) and CLDs (S2B and S2E); of DAPI in nucleus (S2C and S2F).

References

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