Supplementary Information

Probing Polarity and Heterogeneity of Lipid Droplets in Live Cells Using a Push-Pull Fluorophore

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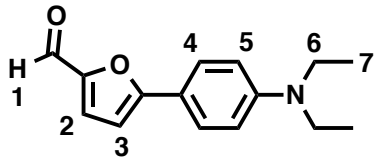
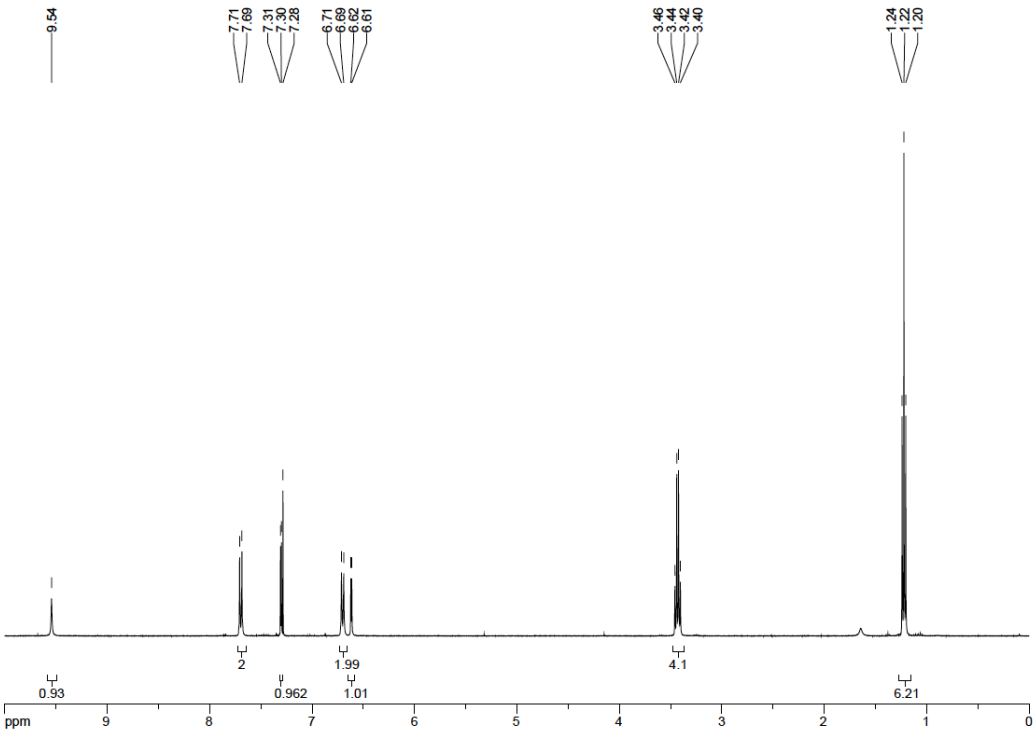
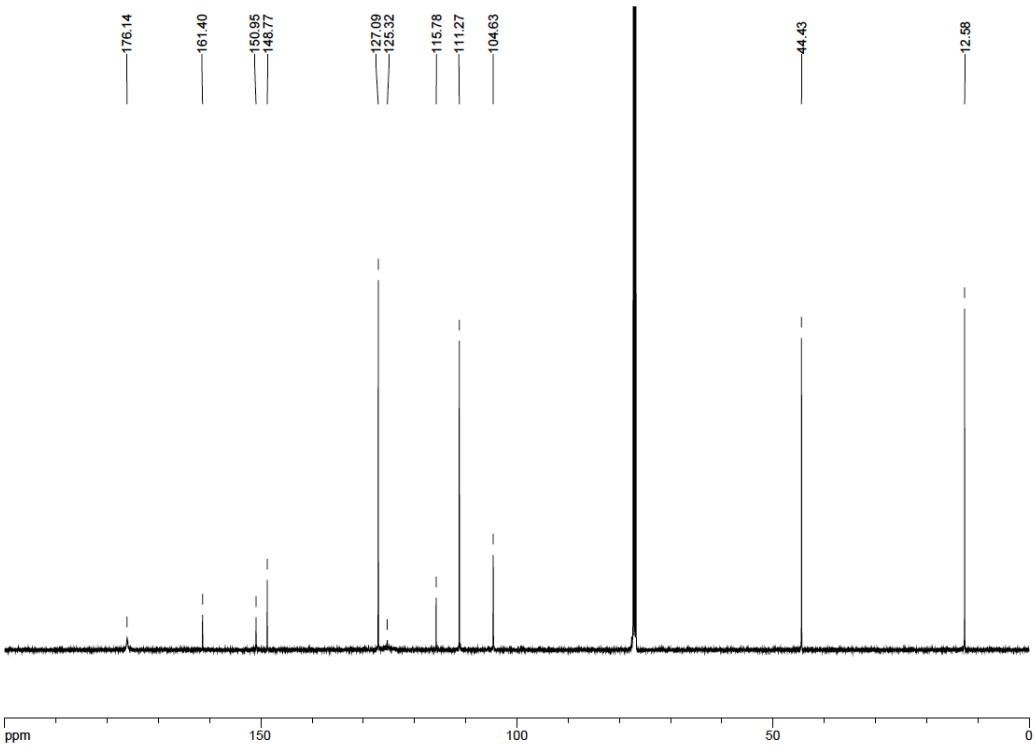
Synthesis and characterization of DAF



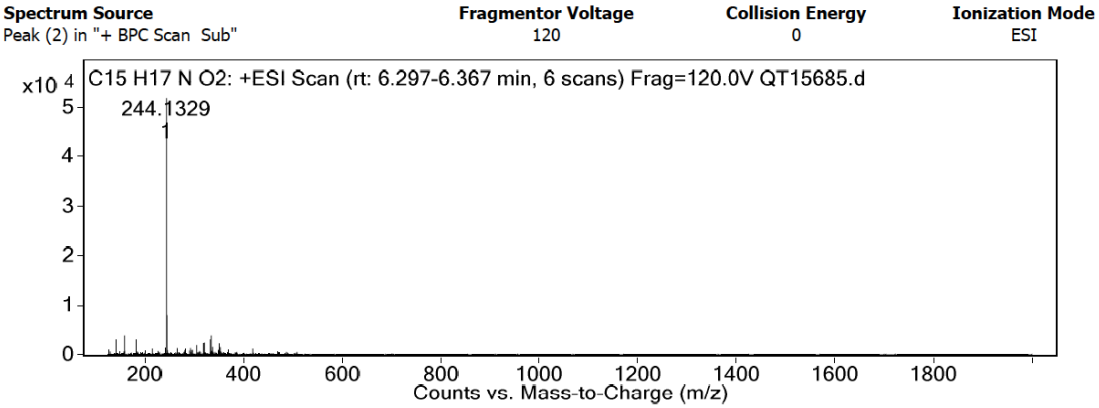
To a solution of (5-formylfuran-2-yl)boronic acid (500 mg, 3.57 mmol) and 4-bromo-*N*,*N*-diethylaniline (814 mg, 3.57 mmol) in dioxane (35 mL) was added an aqueous solution of K_2CO_3 (1.5 g in 6 mL water). The heterogeneous solution was degased 3 times by alternating vacuum and argon. The solution was then warmed up at 95°C until total solubilization and $Pd(dppf)Cl_2$ (255 mg, 0.35 mmol, 0.1 eq) was added. The solution was then allowed to stir under argon overnight at 80°C. The solution was evaporated. The crude was extracted with EtOAc and washed with water. The crude was purified by column chromatography on silica gel (8/2 hepatne/EtOAc) to obtain 140 mg of DAF (Yield=16%) as a yellow powder. Rf= 0.31 (8/2 hepatne/EtOAc). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 9.54 (s, 1H, H1), 7.70 (d, *J* = 9.0 Hz, 2H, H4), 7.30 (d, *J* = 3.8 Hz, 1H, H2), 6.70 (d, *J* = 9.0 Hz, 2H, H5), 6.62 (d, *J* = 3.8 Hz, 1H, H3), 3.43 (q, *J* = 7.1 Hz, H6), 1.22 (t, *J* = 7.1 Hz, H7). ^{13}C-NMR (126 MHz, $CDCl_3$): δ 176.1 (CO), 161.4 (Cq furan), 150.9 (Cq furan), 148.7 (Cq aniline), 127.0 (C4), 125.3 (Cq aniline), 115.7 (C2), 111.2(C5), 104.6 (C3), 44.4 (C6), 12.5 (C7). HRMS (ESI+), calcd for $C_{15}H_{18}NO_2$ $[M+H]^+$ 244.1338, found 244.1329.



^{1}H NMR spectrum of DAF ($CDCl_3$)



^{13}C NMR spectrum of DAF ($CDCl_3$)



HRMS spectrum of DAF

Spectroscopy

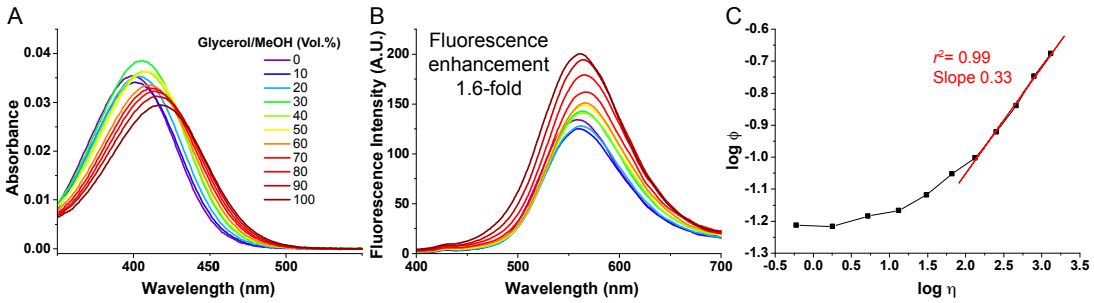


Figure S1. Absorption (A) and emission spectra (B) of DAF (1 μM) in methanolic solutions of glycerol with increasing viscosity. C is a Förster-Hoffmann plot showing the correlation of quantum yield and viscosity. Excitation was at 380 nm.

Cellular experiments / Imaging

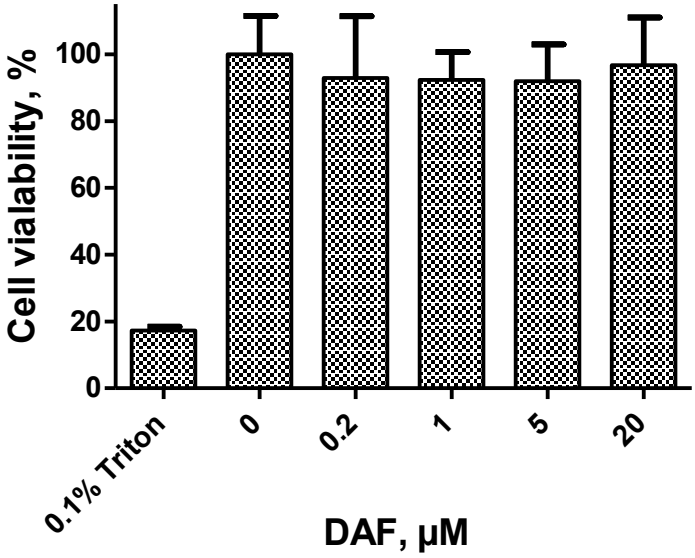


Figure S2. Cytotoxicity assay of DAF after 1 h incubation quantified by MTT assays.

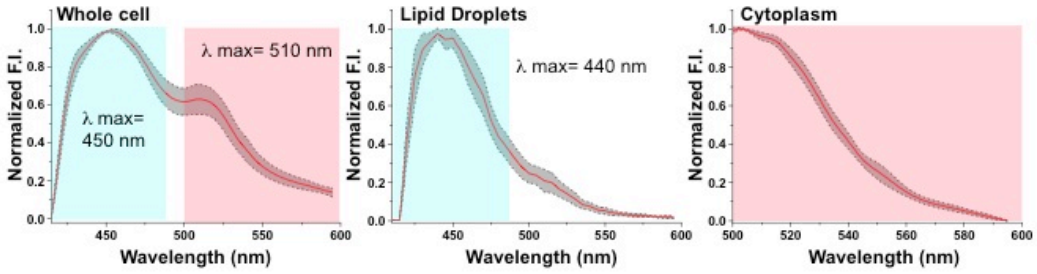


Figure S3. Normalized *in situ* emission spectra of DAF in different regions of interest of live KB cells obtained by lambda scan (5 nm steps) with a laser confocal microscope using 405 nm laser. The gray area is the standard deviation corresponding to ten different LDs measurements (normalized), which were averaged (solid red line).

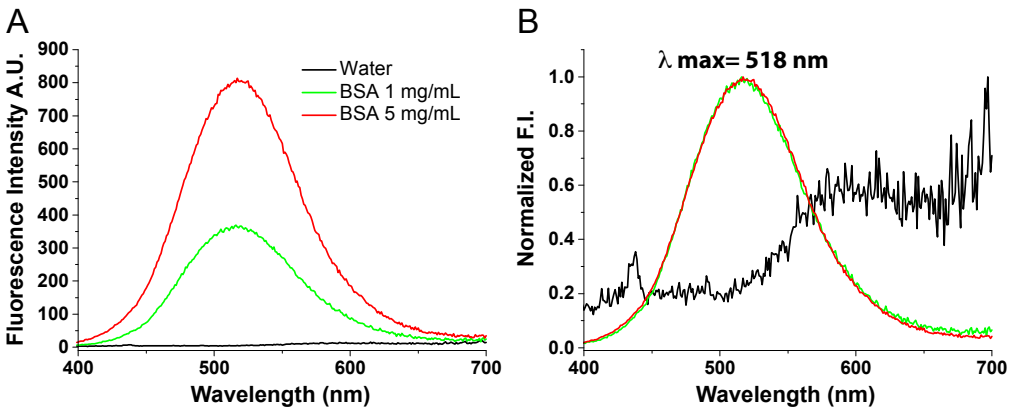


Figure S4. Emission spectra (A) and normalized emission spectra (B) of DAF (1 μM) in water and in the presence of BSA (bovine serum albumin).

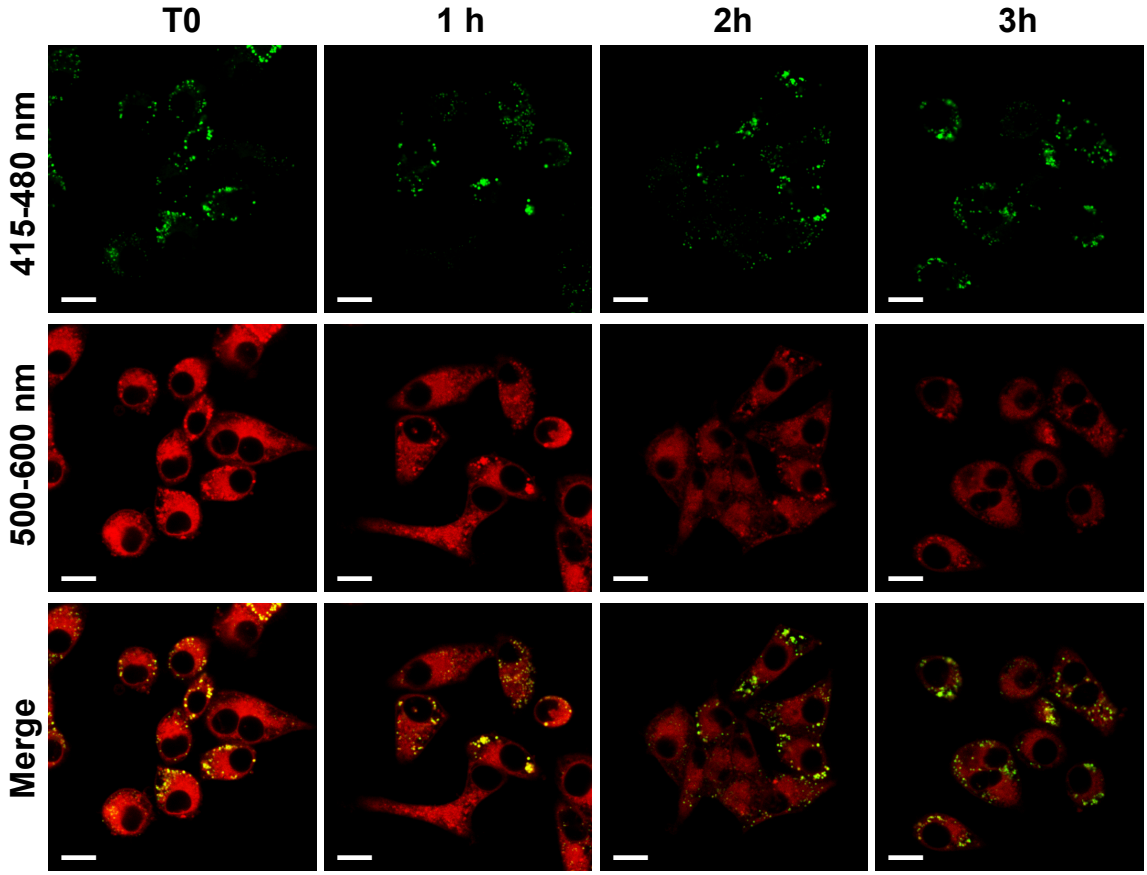


Figure S5. Laser scanning microscopy images of KB cells stained with DAF (2 μM) and imaged at different time of incubation without any washing step. Scale bar is 15 μm.

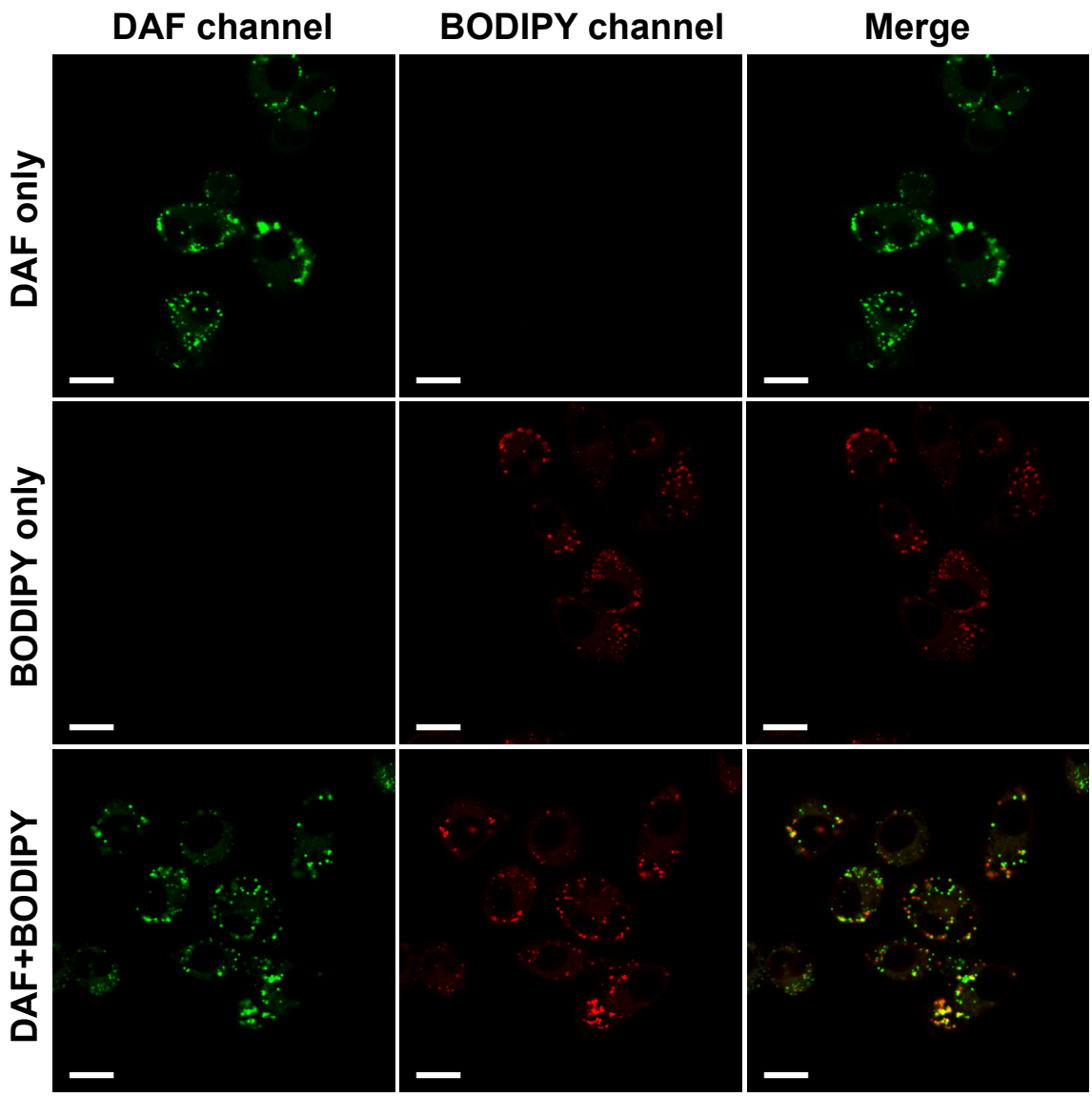


Figure S6. Verification of cross-talk phenomena for the colocalization experiments in live KB cells. DAF was used at 2 μM and BODIPY 493/503 at 200 nM. DAF Channel: λ_{Ex}= 405 nm, Em: 415-480 nm. BODIPY channel λ_{Ex}= 488 nm, Em: 495-550 nm.

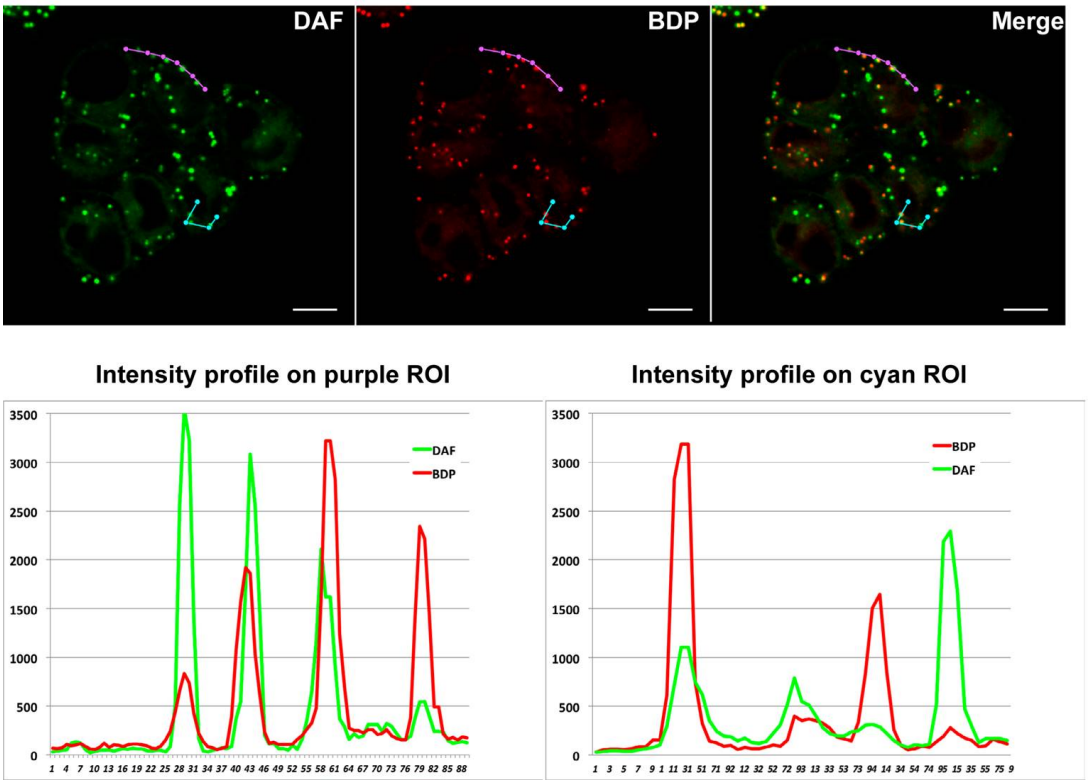


Figure S7. Intensity profile of lines crossing LDs stained with BODIPY 493/503 and DAF shwong the heterogeneous staining of both dye.

Details on colocalization experiments:

Colocalization methods are traditionally divided into pixel-based methods that measure global correlation coefficients from the overlap between pixel intensities in different color channels, and object-based methods that first segment molecule spots and then analyze their spatial distributions with second-order statistics.

Here we first analyze correlation between DAF and BODIPY intensity thanks to the conventional Pearson Coefficient analysis. This is based on the correlation of intensity of the fluorophores in the 2 channels. Pearson coefficient can range from -1 (anti-correlation) to 1 (correlation) passing by zero (no correlation) (see cytometry part 1 for review)[1]. Here, by analyzing 6 pictures, we measured a Pearson coefficient of 0.6995+-0.0154 which indicate a pretty good correlation between DAF and BDP intensity. The intensity profiles showed in figure S7, indicate that most of the LDs harbors both DAF and BDP staining (see intensity profile for purple ROI). However, some LDs are labeled solely with DAF or BDP (see intensity profile for cyan ROI).

Using object based methods, we could segment DAF and BDP spots using wavelets analysis. Those green and red spots could then be analyzed with our Icy SODA plugin. SODA stands for Statistical Object Distance Analysis, it is using Ripley's function to analyze the spatial distribution of the green and red spots (Lagache et al. Nat comm 2018). SODA analyses their relative positions rather than their fluorescence correlation or overlap. By using the center of mass of each spots as coordinates it analyses spatial distribution of green and red dots. This calculation allows identifying green objects, which are statistically coupled to red objects, from those which are just close because of random distribution. Here using SODA analysis, we found that 59% of DAF positive LDs (n= 755) were found to be associated with BODIPY 493/503, and conversely 62% of BODIPY 493/503 positive LDs (n=729) were associated to DAF. This association was statistically highly significant (p value 10^{-216}). Those

data indicate that roughly 60 % of the LDs are labeled with both dyes DAF and BDP. However there are still case, where DAF labeling is alone and vice versa, indicating that LDs is a highly heterogeneous population that can be labeled to a certain extend depending on the sensitivity of the dye used to label them.

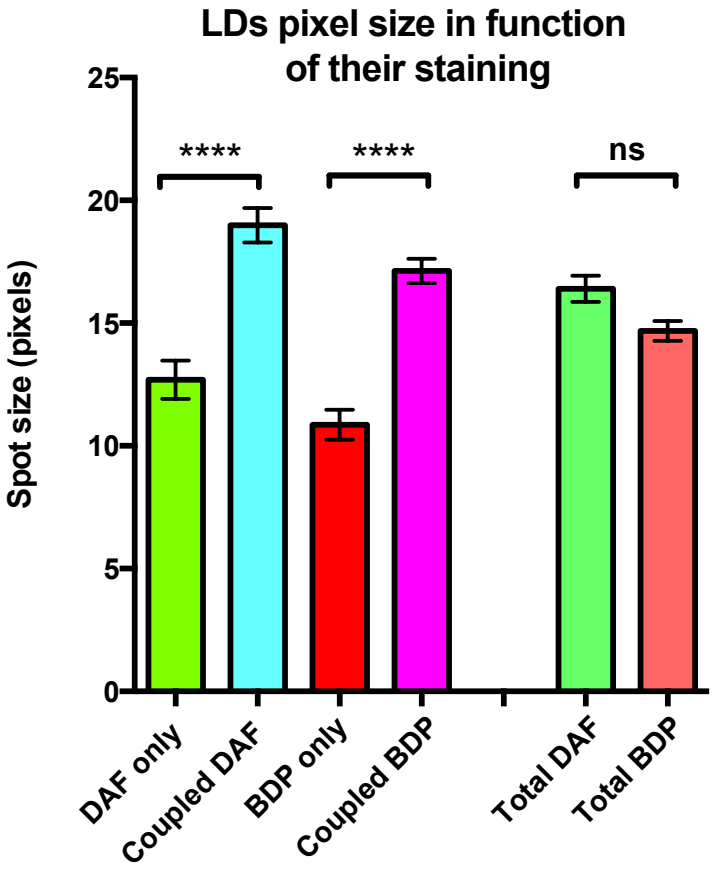


Figure S8. Morphometric analysis of DAF spots size regarding their association with BODIPY staining. When analyzing the total populations of DAF or BODIPY spots, no significant difference in spot size appeared (ns, on the right). However, when comparing isolated DAF spots or DAF spots stained also for BODIPY, we can note that the mean size of the isolated spots (green for DAF, Red for BODIPY) are significantly smaller than the DAF spots containing both dyes (cyan for DAF, pink for BODIPY, Mann and Whitney test, $p<0,0001$). This indicates that bigger LD tends to be labeled with both dyes, whereas smaller one can be either DAF or BODIPY positive.

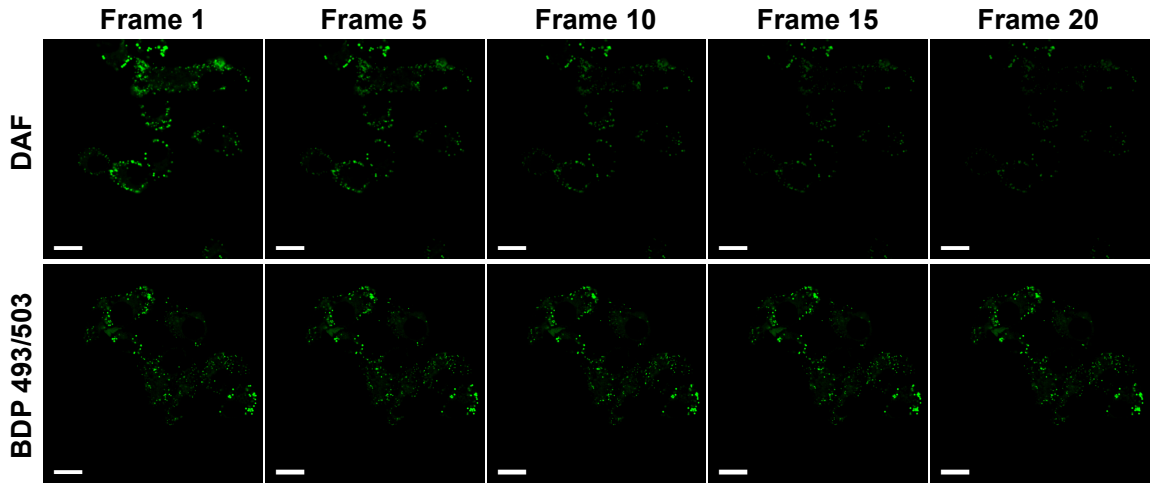


Figure S9. Comparison of the photostability between DAF (2 μM) and BODIPY 493/503 (1 μM) in live KB cells imaging. DAF was 2 times more concentrated than BODIPY as it has a twice-lower extinction coefficient. Cells were continuously irradiated and the maximum signal at frame 1 was set at the limit of the saturation for both dyes. DAF Channel: λ_{Ex}= 405 nm, Em: 415-480 nm. BODIPY channel λ_{Ex}= 488 nm, Em: 495-550 nm.

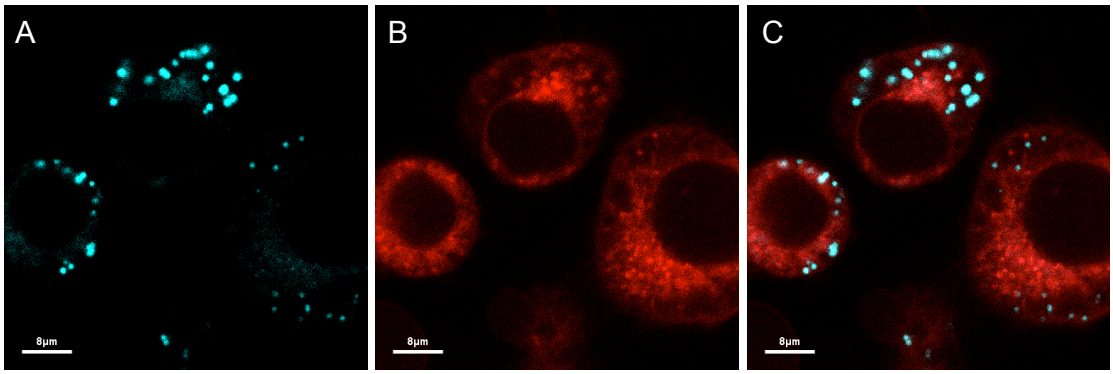


Figure S10. Laser scanning confocal images of fixed (PFA 4%) KB cells incubated for 1 h with DAF (2 μM). Excitation wavelength was 405 nm. A is the blue channel (415-480 nm), B is the red channel (500-600 nm), C is the merge of the blue and red channels.

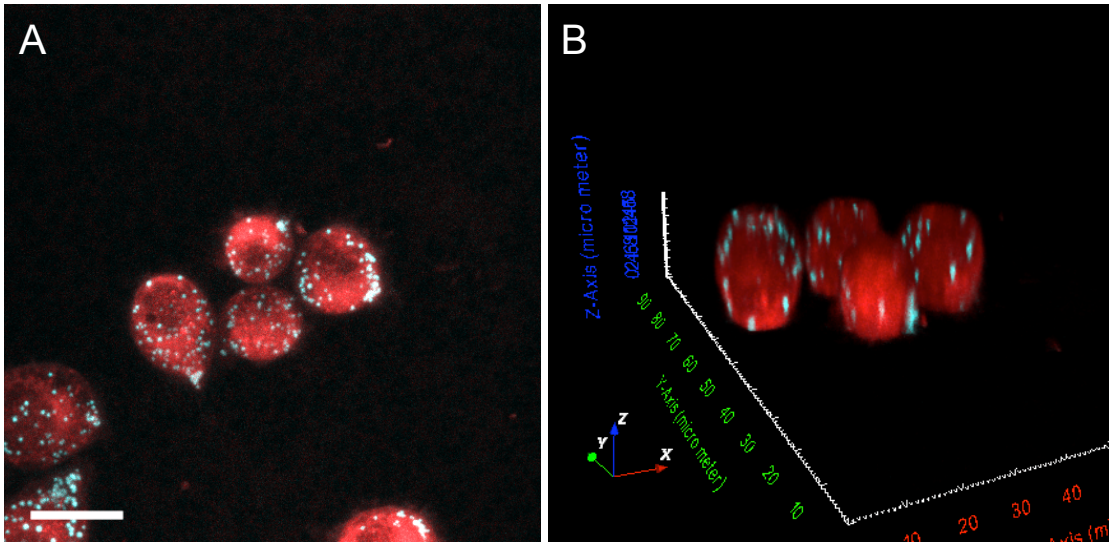


Figure S11. Laser scanning confocal images of fixed (PFA 4%) KB cells incubated for 1 h with DAF (2 μM) using two channels: Blue channel (415-480 nm) and red channel (500-600 nm), excitation wavelength was 405 nm for both channels. A is the maximum projection image of a 67 Z stack images (0.3 μm Z-height each, total height 20 μm) and B is the corresponding 3D image of the 4 cells in the center. Scale bar in A is 20 μm. Background noise in A is due to the first image taken at the surface where the noise is high.

References:

(1) Lagache, T.; Sauvonnet, N.; Danglot, L.; Olivo-Marin, J.-C. Statistical Analysis of Molecule Colocalization in Bioimaging. *Cytometry A* **2015**, *87* (6), 568–579.