Electronic Supplementary Material (ESI) for Nanoscale.

Boron dipyrromethene (BODIPY) Functionalized Carbon Nano-Onions for High Resolution Cellular Imaging.

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Supporting Information

Content:

Spectroscopy: Absorption spectra were recorded on a Varian Cary 300scan UV-vis spectrophotometer or on a Varian Cary 5000 UV-vis-IR spectrophotometer. Corresponding fluorescence spectra were taken on a Varian Cary Eclipse fluorescence spectrophotometer in 1.00 x 1.00 cm quartz glass cells.

Fluorescence quantum yields: Φ_F of **2** in the indicated solvents were determined by the comparative method of Williams *et al*.[S1] and fluorescence quantum yields were calculated using the following equation,

$$\Phi_x = (\Phi_{st}) \, (Grad_x / Grad_{st}) \, (\eta^2_x / \eta^2_{st})$$

where *st* and *x* denotes the standard and test respectively, while Φ is the fluorescence quantum yield. *Grad* is the gradient obtained from the plot of integrated fluorescence intensity *vs.* absorbance of the dye at the excitation wavelength. η represents the refractive index of the used solvents. The fluorescence quantum yields were measured relative to **1** with $\Phi_{St} = 0.64$ in toluene at an excitation wavelength of 485 nm.[S2]

ATR FTIR spectroscopy: FTIR spectroscopic studies were carried out on a Bruker Vertex 70v FTIR spectrometer equipped with a Platinum ATR accessory.

Raman spectroscopy: Raman spectra were measured on a Horiba Jobin Yvon HR 800 UV LabRam Raman microscope. For the Raman measurements, the samples were excited with a built-in 632 nm laser. The samples were deposited by adding the dry compound to a drop of methanol on the glass slide. The slides were dried in air for two hours.

NMR spectroscopy was performed on a Bruker Avance III 400 MHz system (400.13 MHz for ^{1}H and 100.62 MHz for ^{13}C) in $CDCl_3$, purchased from Sigma-Aldrich.

High-resolution mass spectrometry (HRMS): The Accurate Mass measurements (HRMS) were performed on a Waters SYNAPT G2 High Resolution Mass Spectrometry instrument equipped with an Electrospray Ionization interface and coupled to a Waters ACQUITY UPLC. Electrospray ionization in positive mode was applied in the mass scan range 50-1200

Da. The analysis were performed on a Waters ACQUITY UPLC BEH C18 column 100 x 2.1 mm ID (particle size 1.7 μm) with an in-line filter. The mobile phase was 0.1% formic acid in H_2O and 0.1% formic acid in acetonitrile.

Dynamic light scattering (DLS) and Zeta-potential: DLS measurements were performed on the Malvern Nano-ZS instrument operating in backscattering (173°) mode and analyzed with the proprietary software Zetasizer, with automatic selection of the optimal detector position and number of independent measurements. PBS pH 7.4 was chosen as mimic of the DMEM buffer used for biological studies and, by extension, of biological fluids. About 0.3 mg of CNO sample were weighted and dispersed in DMSO to a final concentration of 1.0 mg/mL and intensively sonicated for 1 hour at 37 Hz. 12μL of the dispersion were then diluted in PBS to achieve a final concentration of CNOs of 10 μg/mL. The suspension was then sonicated at 37 Hz for additional 20 minutes and particle size was suddenly measured. Z-potential measurements were performed on the same apparatus using the disposable proprietary Z-potential cuvettes. In order to prevent alteration on the properties of particles' surface due to DMSO, CNOs samples were directly dispersed in a low ionic strength phosphate buffer (0.01M, pH 7.4) to a final concentration of 20 μg/mL and intensively sonicated (37 Hz, 2 hours) prior to measurements.

Transmission electron microscopy: For TEM investigations, all the CNO samples were suspended in spectroscopic grade methanol, mildly sonicated and deposited onto Cu grids covered with a holey C film. BF-TEM imaging was performed on a Jeol JEM-1011 instrument equipped with a thermoionic tungsten source and operated at 100 kV. HR-TEM imaging and EEL spectroscopy analysis were carried out on a Jeol JEM 2200FS equipped with a Schottky FEG source operated at 200 kV, a CEOS image aberration corrector and an in-column energy filter (Ω-type). The EEL spectra were collected on areas (250 nm diameter) of CNO aggregates suspended on holes, in order to avoid contributions from the amorphous support film. The spectra were acquired in diffraction mode, with a collection semi-angle of

5.5 mrad, an energy resolution of 1.1 eV and a dispersion of 0.3 eV/pixel. The presented spectra were obtained by background subtraction and plural scattering deconvolution from the raw spectra.

Atomic force microscopy: Topographic images were collected in non-contact mode with a Park XE-100 AFM. Two types of tips were used, both of them having nominal resonance frequency of 330 kHz, nominal force constant of 42N/m and a reflectance aluminum coating on the back. Figure 5a was obtained with a tip featuring a nominal apex radius of 10 nm (PPP-NCHR), while Figure 5b was taken with a tip having a nominal apex radius of 2 nm (SSS-NCHR). Images were collected with the XE software and data were processed with the XEI and Origin software. Samples were prepared by dispersing **BODIPY-CNO**s in methanol to a final concentration of about 0.01 mg/mL. Samples were then bath-sonicated (sonicator from Falc, LCD Series) for 1.5 hours at 59 kHz at RT. 10μL of the dispersion were then drop casted on freshly-cleaved mica (whose surface was about 3.75 cm^2) then dried overnight at 80° C in a Binder (ED 53) oven.

X-ray crystallography: A good quality orange-colored crystal of the dimension 0.20x0.15x0.05 mm was used for the determination of cell parameters and for the whole data collection. The X-ray data were collected at 100 K on a Bruker X8 Prospector APEX-II/CCD diffractometer, using CuKα radiation (λ=1.54050). Crystal data: C_{26} H_{23} N_2 O_2 F_2 B; Mr = 444.27; orthorhombic F, Fdd2; a = 18.4695(18) Å, b = 43.010(4) Å, c = 10.9507(11) Å, α = β = γ = 90°; Z = 16; V = 8699.0(15) Å3; μ = 0.80 mm-1.

The structure was solved by direct methods and refined with SHELX-L97.[S3] All non-hydrogen atoms were refined anisotropically by the full matrix least-squares refinement method. The model converged to a final R value of 0.0555, [wR(F2)= 0.154, S=1.12 for 2757 reflections with [I > 2σ(I)]. The final difference map was featureless ($\Delta_{\rho max}$ = 0.28 $eÅ^{-3}$, $\Delta_{\rho min}$= -0.29 $eÅ^{-3}$)

Thermogravymetric analysis: TGA was conducted on a TA Q500 analyzer, using a Pt pan as sample holder. The measurement was performed in air using a heating rate of 10 °C/min, After equilibrating the sample at 30°C for 5 min and then at 100°C for additional 20 min, the sample weight was monitored until 900 °C have been reached by the furnace.

Figure S1. Numbering Scheme of the molecular structure of **2**. Hydrogen atoms omitted for clarity.

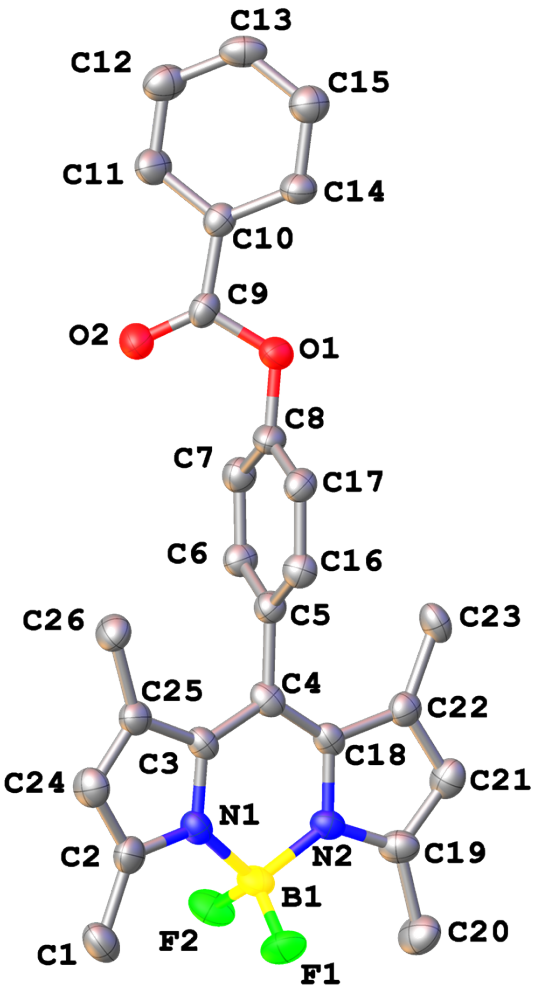


Table S1. Parameters for data collection, structure solution and refinement for **2**.

CCDC #	1015701
Chemical formula	C_{26} H_{23} N_2 O_2 F_2 B
M_r	444.27
Crystal system, space group	orthorhombic F, *Fdd2*
Temperature (K)	100
a, b, c (Å)	18.4695(18), 43.010(4), 10.9507(11)
α, β, γ	90.00, 90.00, 90.00
V (Å^3)	8699.0(15)
Z	16
Radiation type, λ (Å)	CuK_α , 1.54
μ (mm^{-1})	0.80
Crystal size (mm)	0.20x0.15x0.05
<u>Data collection</u>	
Absorption correction	Multi-scan
No. of measured, independent and observed [I > 2σ(I)] reflections	6167, 2757, 2283
R_{int}	0.043
<u>Refinement</u>	
R[F^2 > 2σ(F^2)], wR(F^2), S	0.055, 0.154, 1.12
No. of reflections	2757
No. of parameters	303
$\Delta_{\rho max}$, $\Delta_{\rho min}$ (e Å^{-3})	0.28, -0.29

Figure S2. Baseline corrected solid state ATR FTIR spectrum of **2**.

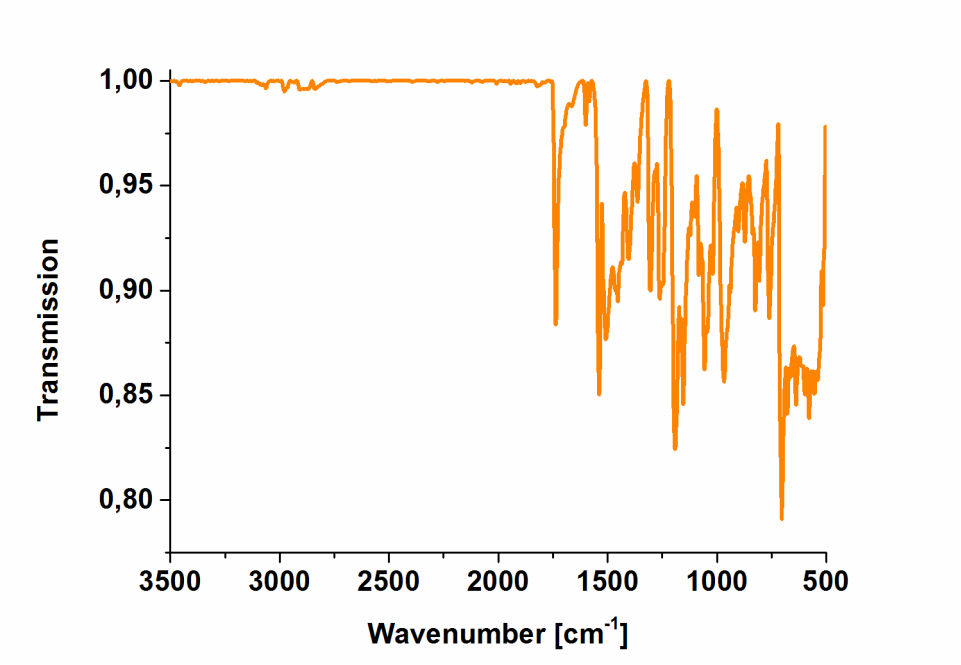


Figure S3. Plots of the thermogravimetric analysis (top) and the corresponding weight loss derivative (bottom) of **p-CNO**s (black), **benz-CNO**s (red) and **BODIPY-CNO**s (blue).

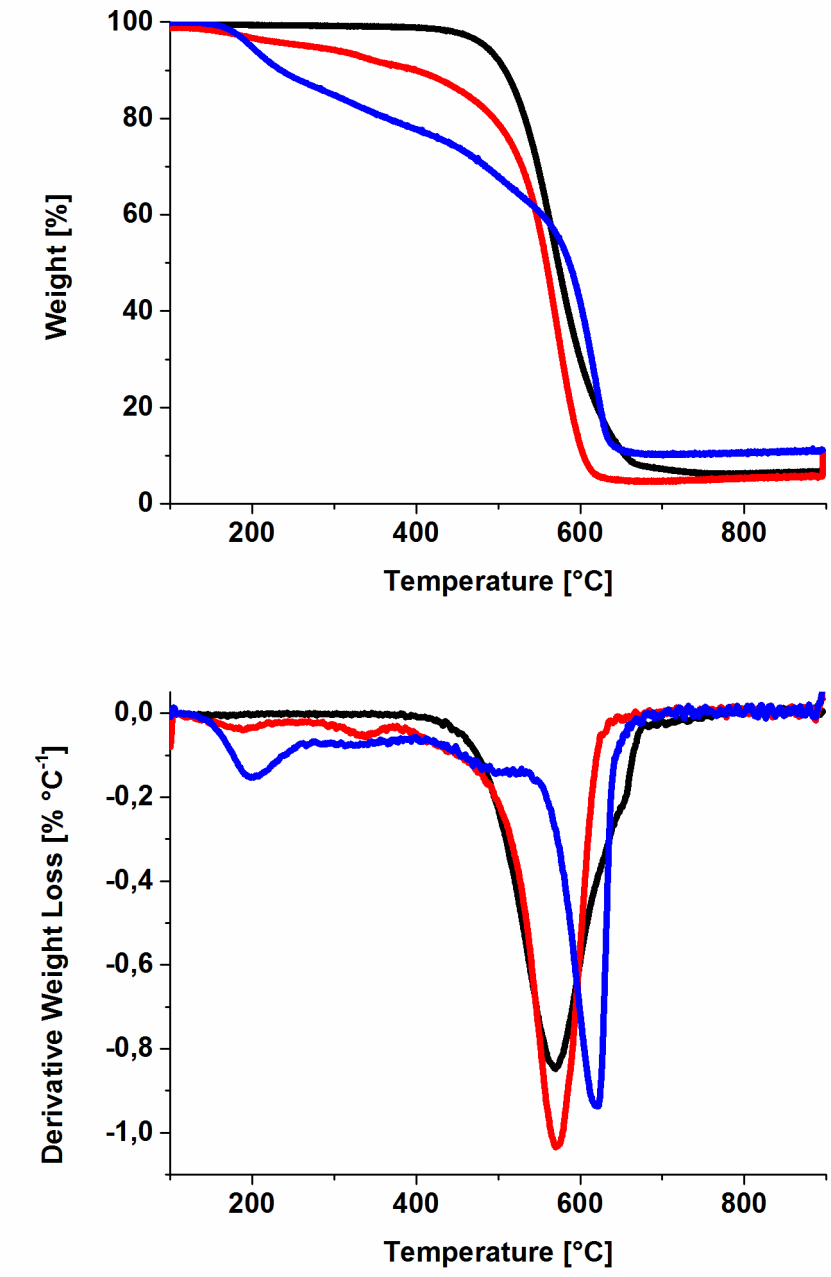


Table S2. DLS and Z-potential data.

Results of DLS measurements: effective hydrodynamic diameter

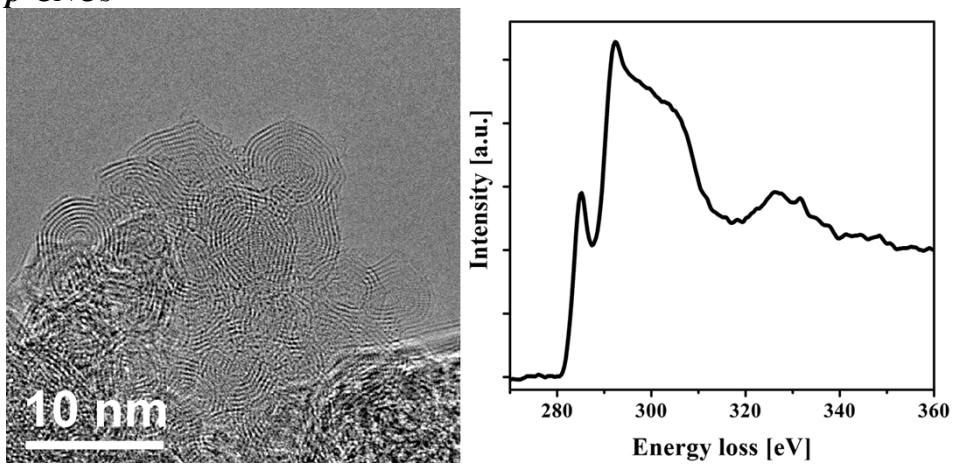
	benz-CNO	**BODIPY-CNO**
Average hydrodynamic diameter ± sd	195.0 ± 32,95 nm (66,1%) 516.8 ± 133,1 nm (33,9%)	110.8 ± 16,41 nm (42,7%) 428.1 ± 93,5nm (57,3%)

Z-potential

	benz-CNO	**BODIPY-CNO**
Z-potential (mV) ± sd	-40.0 ± 5.09	-23.0 ± 3.91

Figure S4. HR-TEM and EELS of **p-CNO**s and **benz-CNO**s. (Left) HR-TEM images of **p-CNO**s and **benz-CNO**s in aggregates partly suspended on holes (inter-shell spacing 3.4 Å). (Right) Single-scattering EEL spectra at the carbon K-edge collected from aggregates suspended on vacuum.

p-CNOs



Benz-CNO

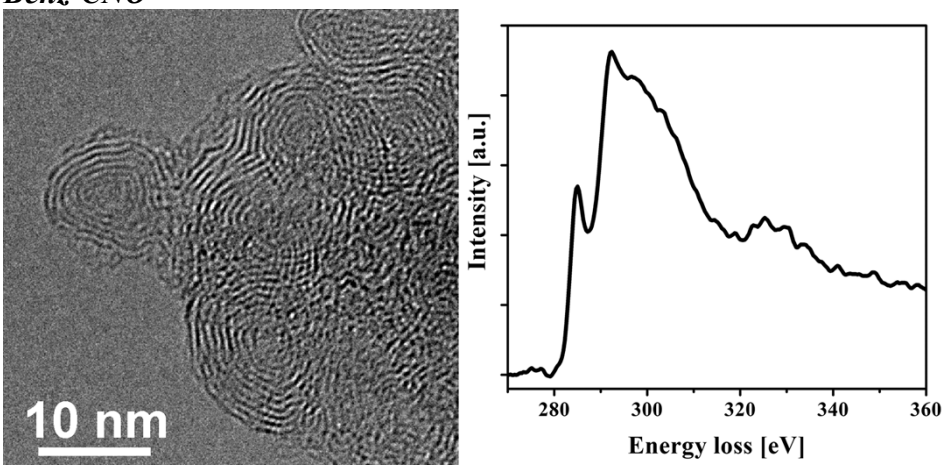


Figure S5. Colocalization of CNOs with lysosomes. (A) Confocal imaging of subcellular localization of **BODIPY-CNO**s after 48 h of incubation in MCF-7 cells; (a) green fluorescent **BODIPY-CNO**s, (b) lysosomes stained with Lysotracker Red probe, and (c) merged images. (B) Colocalization analysis of **BODIPY-CNO**s with lysosomes. Colocalization mask (white) showing the overlapping regions of (a) green and (b) red signals; (c) scatter plot of green intensities versus red intensities, with the pixels in the middle area of the plot representing the colocalization signals. Pearson's correlation coefficient = 0.7892.

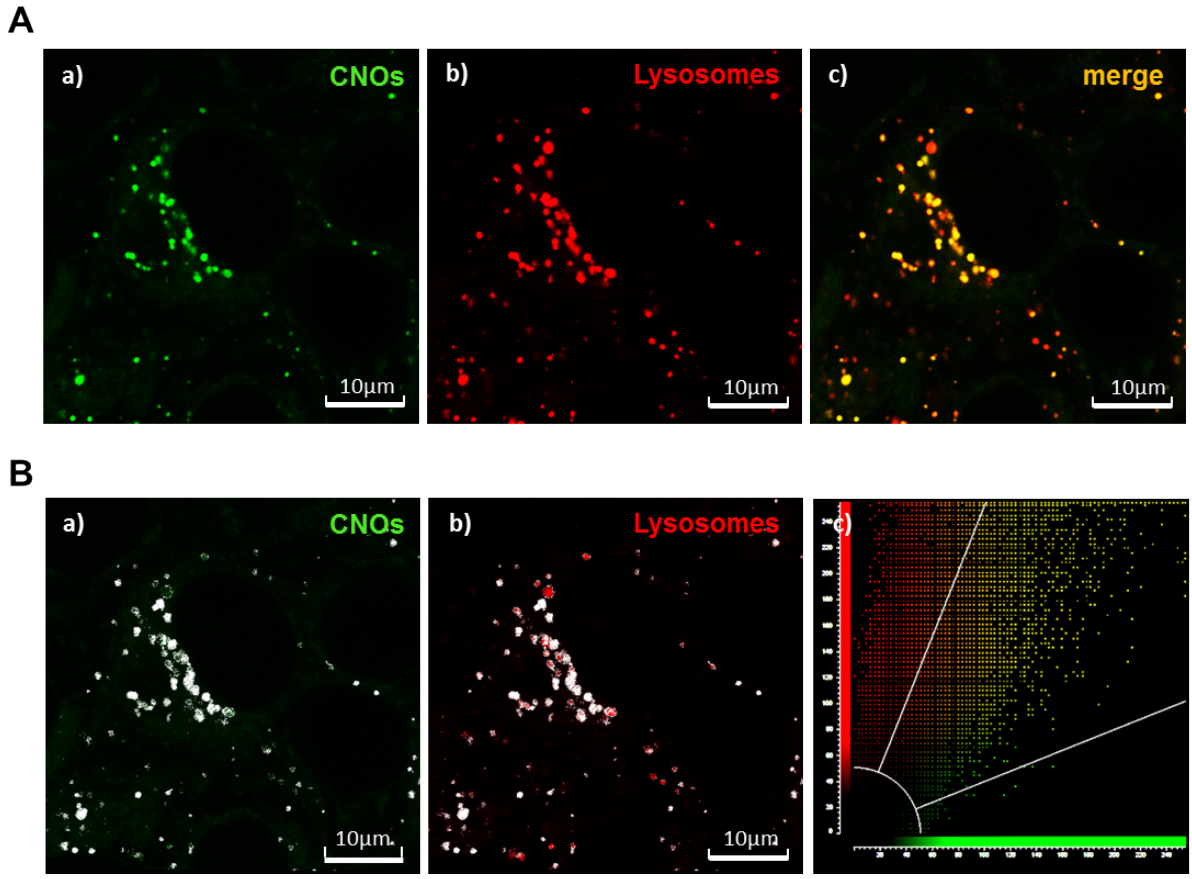
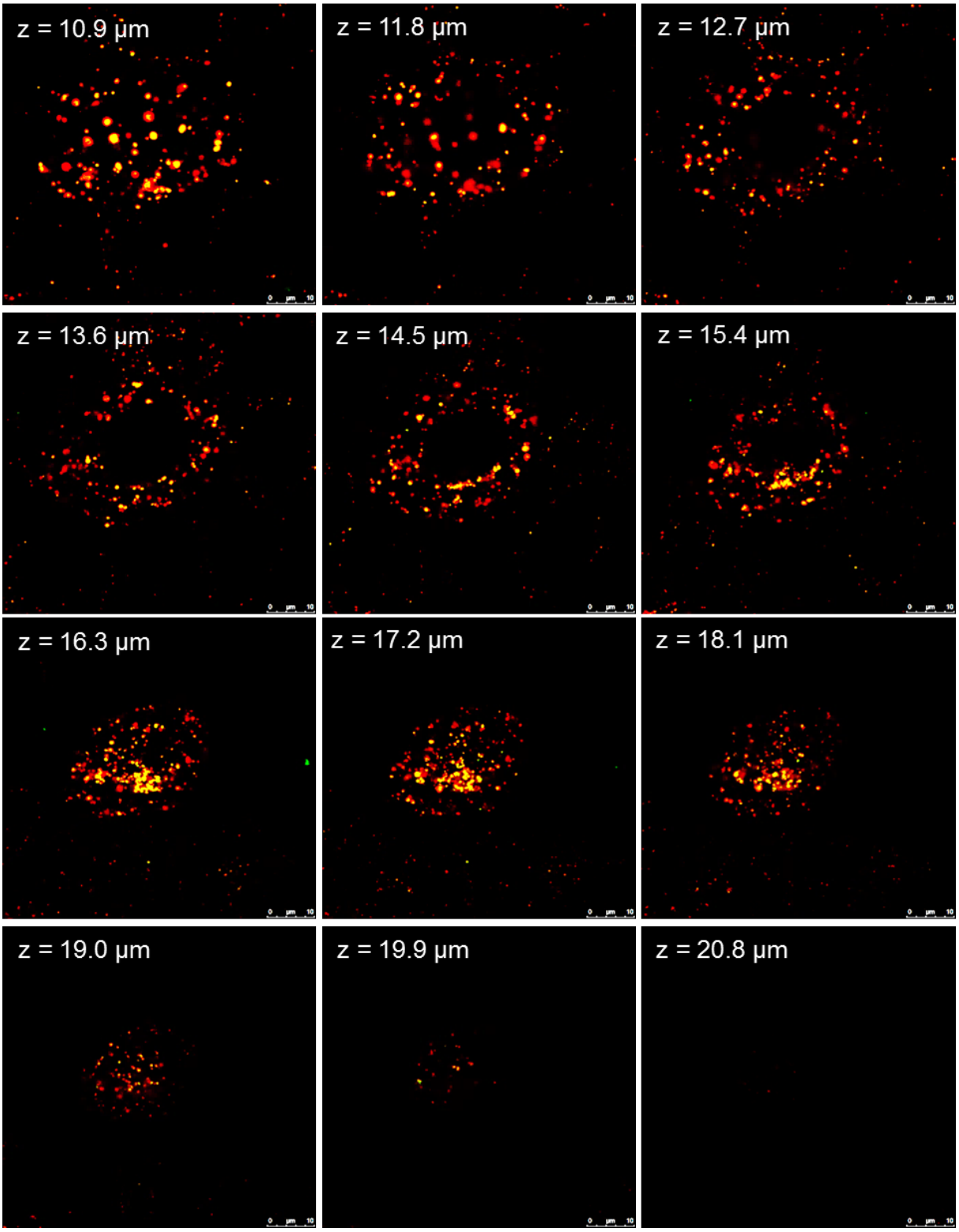


Figure S6. Z-stack confocal images of fluorescent **BODIPY-CNO**s and lysosomes in MCF-7 cells. Representative sequence of frames of optical sectioning, showing the colocalization of fluorescent **BODIPY-CNO**s (green) and lysosomes (red). Z-stacks were acquired from the bottom to the top of the cell. Notice the almost exclusive overlap between green and red signals over the entire volume of the cell occupied by lysosomal vesicles.



Movie M1. Movie of Z-stack series of fluorescent **BODIPY-CNO** and lysosomes in MCF-7 cells.

submitted together with this manuscript

Literature:

[S1] A. T. R. Williams, S. A. Winfield, J. N. Miller, *Analyst* **1983**, *108*, 1067.

[S2] T. Lazarides, S. Kuhri, G. Charalambidis, M. K. Panda, D. M. Guldi, A. G. Coutselos, *Inorg. Chem.* **2012**, *51*, 4193.

[S3] G. M. Sheldrick, *SHELXS-97 and SHELXL-97, Programs for the Solution and Refinement of Crystal Structures*, **1997**, University of Göttingen, Göttingen, Germany.