Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Specific HOCl Tracking Across Diverse Cellular Organelles in Real time using a Superresolution Microscopy Probe

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Experimental details:

Materials & Methods:

All commercial reagents were procured from suppliers, were used as received without further purification. Solvents were dried as and when required by using standard procedures. ·OH radical was generated by Fenton reaction of $FeSO_4$ and H_2O_2 and singlet $^1O_2$ was generated by the reaction of H_2O_2 and NaOCl. ^1H and ^{13}C NMR spectra were recorded on Bruker 400/500 MHz FT NMR (Model: Advance-DPX 400/500) using TMS as an internal standard. All the Fluorescence measurements were carried out on *PTI* Quanta Master™ Steady State Spectrofluorometer. ESI-Mass spectra were recorded on JEOL JM AX 505 HA mass spectrometer. UV-Vis spectra were recorded using Shimadzu UV-1800 spectrometer. Quantum yield was recorded using standard methods and rhodamine B as standard. All the Structured Illlumination Microscopy (SIM) and Wide Field Fluorescence Microscopy experiments were performed by using Delta Vision OMX-SIM (GE Health care). The Post processing SIM reconstructions were performed by using Soft Worx software.

Reagents used for the Tissue culture include, Dulbecco's Modified Eagle's Medium (DMEM) with L-glucose and Sodium bi carbonate (Aldrich), Phosphate Buffer Saline (PBS) (Aldrich), Fetal Bovine Serum (Aldrich), Penicillin Streptomycin (Aldrich). Reagents used for sample preparation for Structured Illumination Microscopy (SIM) and Wide Field Fluorescence Microscopy include 4% Paraformaldehyde (PFA) (Aldrich), Vectashield h-1000 (Mounting agent) (Aldrich), 50 mM Ammonium Chloride (Aldrich), Hoechst 33342 (Aldrich), ER Tracker Green (Aldrich), Cytopainter Golgi staining kit (Abcam), Lipopolysaccharide (Aldrich), Sodium Hypochlorite (Aldrich). Other items required for sample preparation include 26 mm X 76 mm Microscopy glass slides, 22 mm X 22 mm (170 ± 5 μm square Cover glasses (Thor labs.)

General Experimental procedure for photo-physical Experiments:

Stock solution of probe **SF-1** (1×10^{-4} M) was prepared in HPLC grade Acetonitrile and the same solution was used for all the studies after appropriate dilution to 5 ml of PBS (pH 7.2) to make the effective ligand concentration of 10 μM. Unless and otherwise mentioned, 10 mM and pH 7.2 solution of aq. PBS buffer was used for all spectroscopic studies. All reactive oxygen species and nitrogen species solutions of 1×10^{-2} M were prepared in PBS having pH

7.2 and used with appropriate dilution. All luminescence measurements were done using λ_{Ex} = 550 nm with an emission slit width of 2/2 nm. For all biological studies we have used 0.6% DMSO in DMEM media.

Synthesis of SF-1:

Mixture of BODIPY core (280 mg, 0.864 mmol), 3,4-Dihydroxy benzaldehyde (119.3 mg, 0.864 mmol), Glacial acetic acid (0.53ml) and Piperidine (0.65 ml) were refluxed in 10 ml toluene in a dean-stark apparatus for 3h. Crude compound was then evaporated under vacuum and then it was purified by silica gel column chromatography using 5% ethyl acetate in dichloromethane to get pink colour **SF-1** (yield= 18%). **^{1}H NMR** (400MHz, CD_3OD, δ ppm, *J* in Hz): 7.50 (3H, m, Ar-H), 7.46 (1H, *J*=15.89 Hz (CH=C)), 7.27-7.25 (2H, m) , 7.23 (1H, d,*J*=16.38 Hz (CH=CH)), 7.14 (1H, d, *J*=1.96 Hz), 6.94 (1H, dd, *J*=1.96 Hz, *J*=8.07 Hz), 6.80 (1H, d, *J*=8.07 Hz) , 6.65 (2H, s) , 6.02 (1H, s), 2.52 (3H, s), 1.39 (3H, s), 1.37 (3H, s). **^{13}C NMR** (400 MHz CD_3OD): 14.56, 14.65, 14.89, 114.24, 116.61, 117.02, 118.81, 121.91, 122.23, 129.55, 130.15, 130.29, 130.32, 132.03, 134.03, 136.41, 138.56, 141.36, 143.13, 144.36, 146.5, 148.52, 155.00, 155.39, (MALDI MS (m/z) :calculated for $C_{26}H_{23}BF_2N_2O_2$: 444.2888; observed 444.3266. Elemental Analysis: Calculated C 70.29, H 5.22, N 6.31; experimentally obtained C 70.38, H 5.12, N 6.42.

^{1}H NMR spectrum of SF-1:

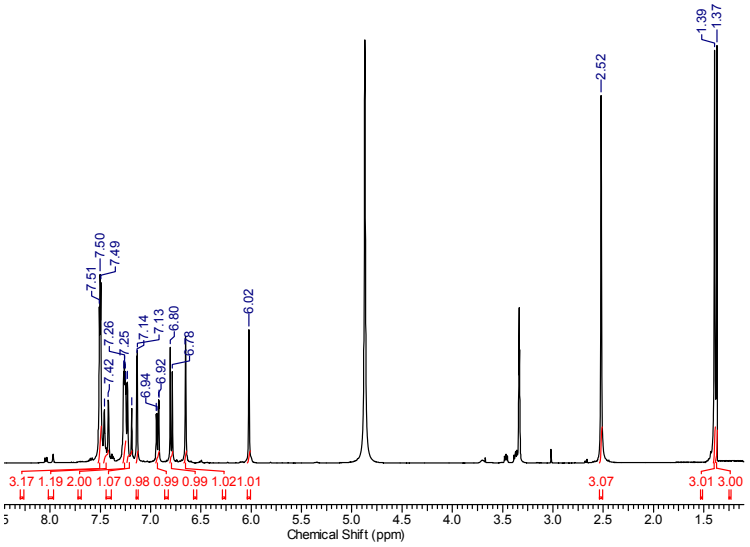

Figure S1. ^{1}H NMR of **SF-1** in CD_3OD.

^{13}C NMR spectrum of SF-1:

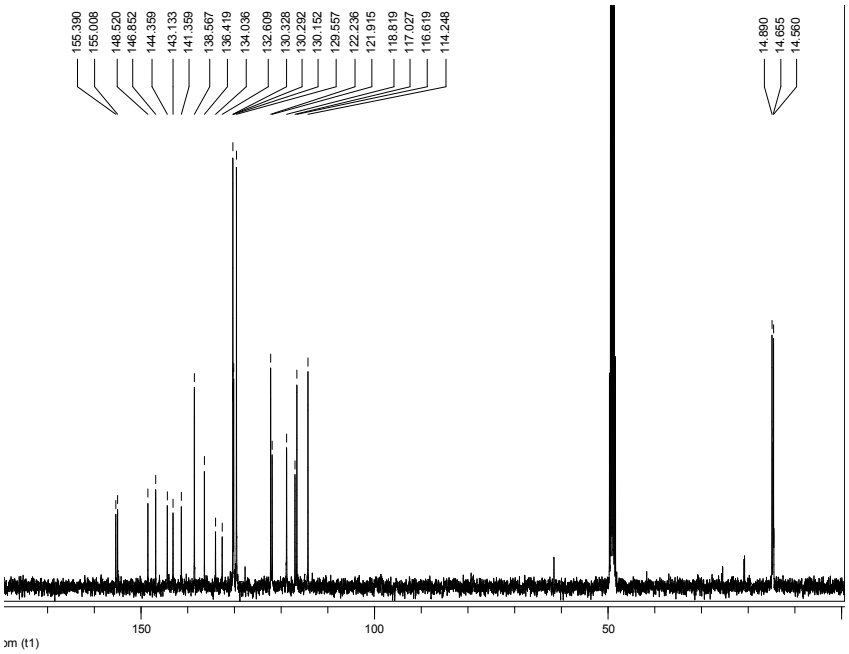

Figure S2. ^{13}C NMR of **SF-1** in CD_3OD.

MALDI-MS spectrum of SF-1:

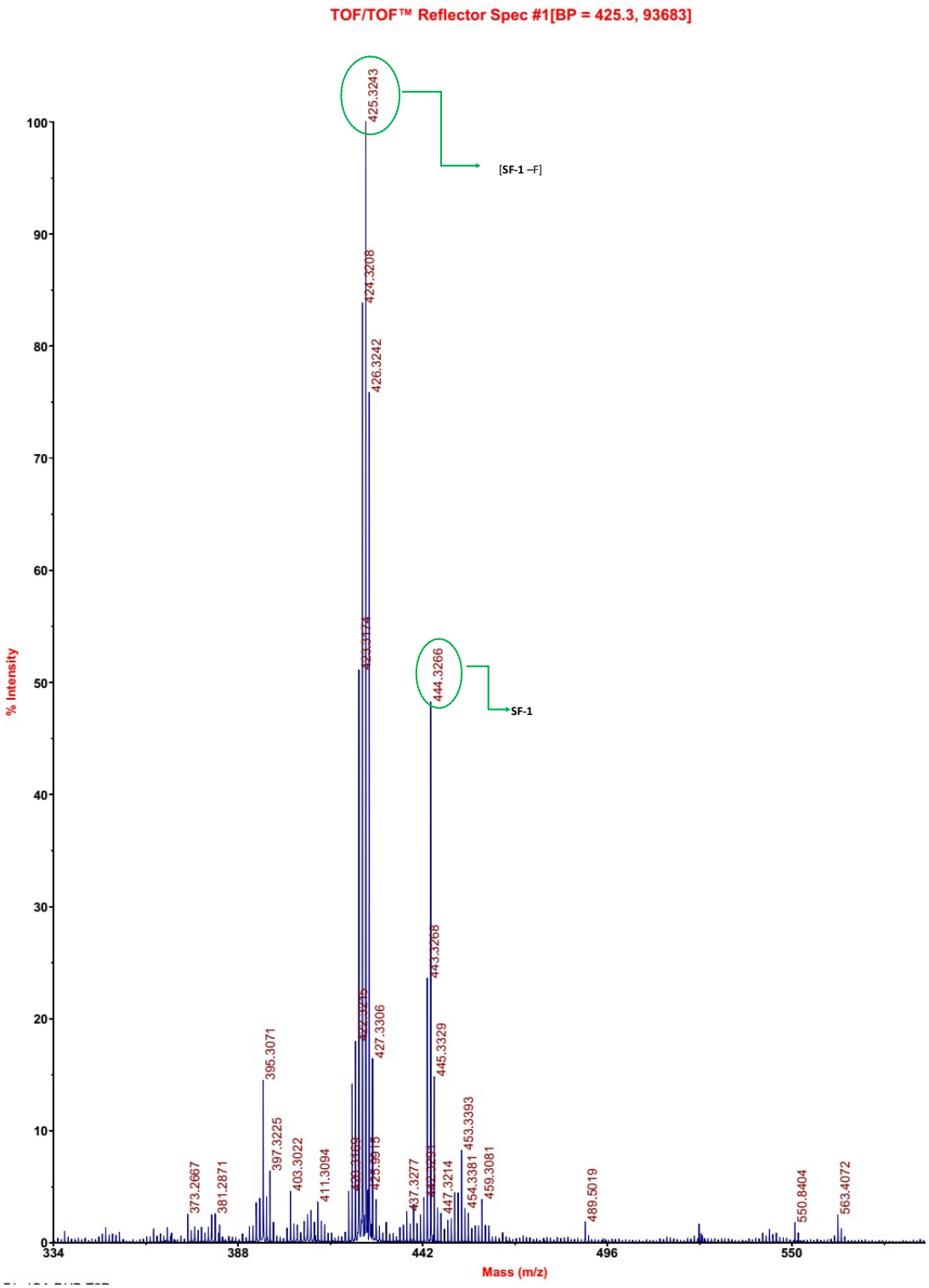

Figure S3. MALDI Ms spectrum of probe **SF-1**. Spectra were recorded using Dithranol (1,8-dihydroxy-9,10-dihydroanthracen-9-one) as the inert matrix using instrument AB SCIEX MALDI TOF/TOFTM 5800.

UV-Vis spectra of SF-1:

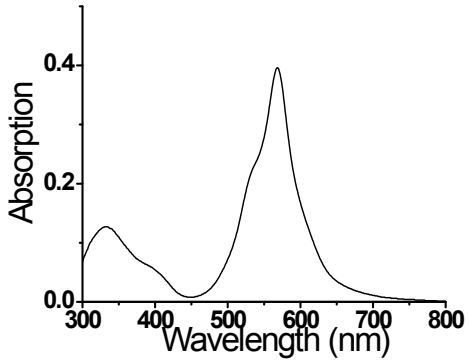

Figure S4. Absorbance spectrum of **SF-1** (10 μM) in Acetonitrile: PBS medium (1:9, v/v) with pH 7.2.

pH response of SF-1:

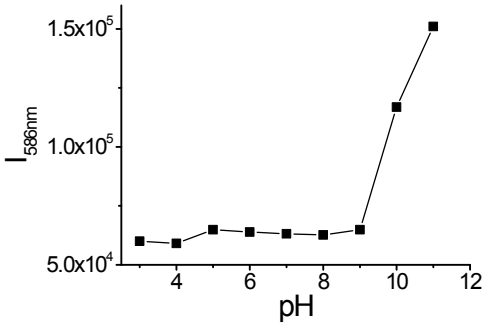

Figure S5.: Change in emission intensity of **SF-1** (10 μM) different pH medium. λ_{Ex} = 550 nm, λ_{Em} = 586 nm.

Selectivity studies of SF-1:

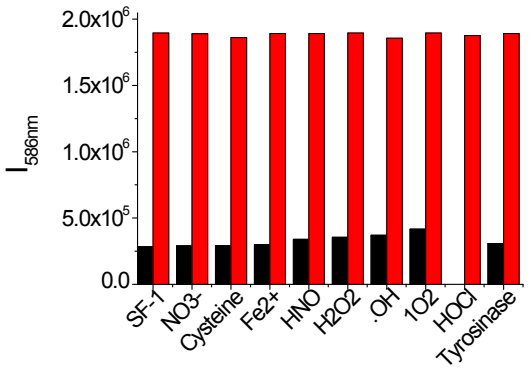

Figure S6: Changes in emission intensity of **SF-1** (10 μM) induced by HOCl (2 mM) in the presence of (2 mM) of other analytes mentioned in the above figure. Red bar and black bar represent emission response in the presence and absence of HOCl, respectively. λ_{Ex} = 550 nm, λ_{Em} = 586 nm.

Time dependent response of HOCl with SF-1:

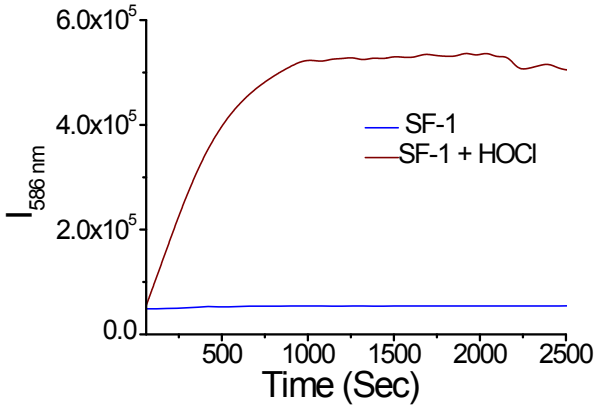

Figure S7. Time dependent emission studies of **SF-1** (10 μM) in absence and presence of 2 mM HOCl in PBS –Acetonitrile medium (9:1, v/v) of pH 7.2 at 37^{0}C. λ_{Ex} = 550 nm. λ_{Em} = 586 nm.

Calculation of detection limit:

The detection limit was calculated based on the fluorescence titration. To determine the S/N ratio, the emission intensity of only **SF-1** probe without addition of HOCl was measured 8 times and the standard deviation of blank measurements was determined. The detection limit (DL) of **SF-1** for HOCl was determined from the following equation:

DL = K * Sb1/S

Where K = 2 or 3 (we took 3 in this case);

Sb1 is the standard deviation of the blank solution;

S is the slope of the calibration curve.

From the graph we get slope = 2.50×10^9, and Sb1 value is 3.59

Thus using the formula we get the Detection Limit = 4.3×10^{-9} M.

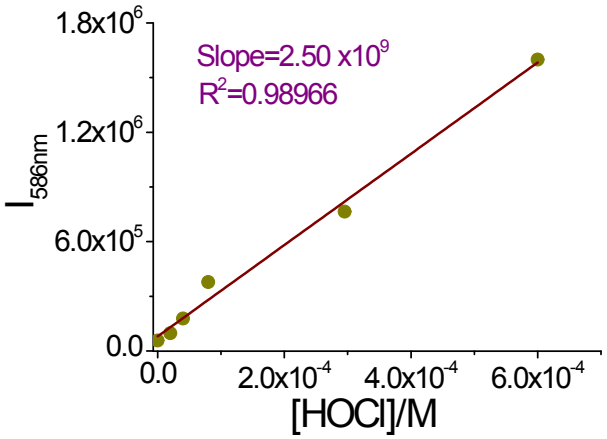

Figure S8. Fluorescence intensity at 586 nm upon addition of HOCl (0 - 0.1 mM) in 10 mM Aq-PBS: CH_3CN (9:1, v/v) at pH 7.4, λ_{Ex} = 550 nm.

MTT assay:

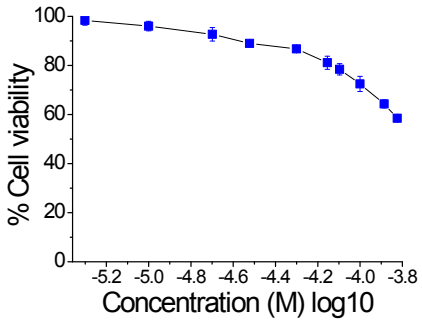

Figure S9. MTT assay to determine the cell viability percentage in presence of **SF-1** in RAW 264.7 cells. IC_{50} was found to be > 150 μM.

The *in vitro* cytotoxicity of **SF-1** on RAW 264.7 cells were determined by conventional MTT (3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) assay. RAW 264.7 cells (5×10^3) were seeded in each well of a 96 well plate and cultured in a 37°C incubator supplied with 5% CO_2. Cells were maintained in DMEM medium, supplemented with 10% Foetal Bovine Serum and 100 Units of Penicillin Streptomycin antibiotics. After 24 hours the cells were treated with different concentrations of the **SF-1** in triplicates for 24 hours. After the treatment, cells were added with 0.5 μg/ml of MTT reagent. The plate was then incubated for 4 hours at 37°C. 100 μL of Isopropyl Alcohol was added to each well. Optical density was measured at 570 nm using Multiskan Go (Thermo Scientific) to find the concentration of the cell inhibition. IC_{50} value has been calculated to be > 150 μM.

The formula used for the calculation of the MTT assay for evaluation of the cell viability was as follows:

Cell viability (%) = (Means of absorbance value of treated group/ Means of absorbance value of untreated control) × 100.

Using the same protocol an MTT assay for evaluation of the cell viability was also done for **SF-2** and its IC_{50} value was calculated to be 110 μM.

Live cell time dependent HOCl detection in RAW 264.7 cells:

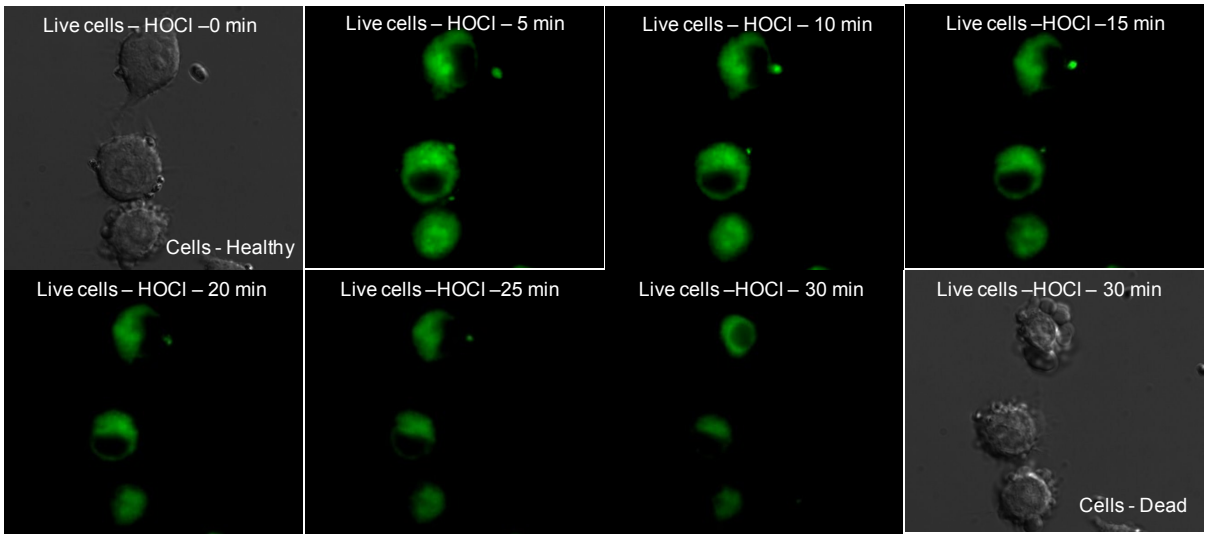

Figure S10. Live cell Wide Field Fluorescence Microscopy Images of **SF-1** (10 μM) detecting externally added HOCl (20 μM) with time.

A comparison of conventional wide-field and SIM images:

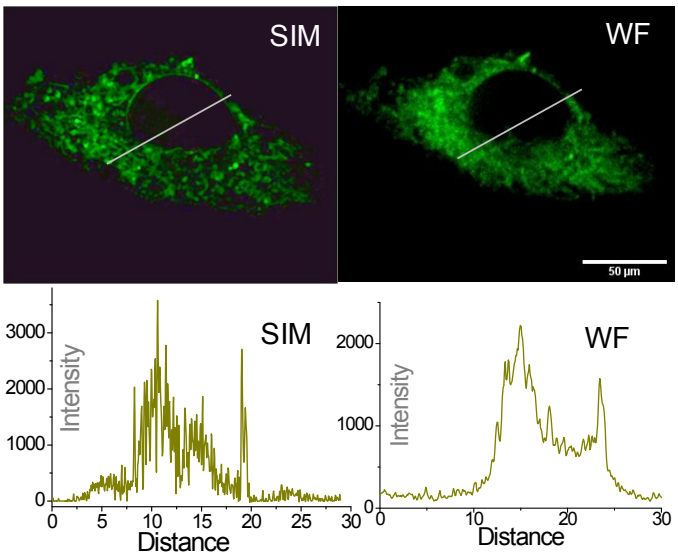

Figure S11. A comparison of conventional Wide-field and SIM images and emission intensity profiles for Fixed cells stained with **SF-1** after external addition of HOCl (Pseudo Colouring has been employed in this and the following images); First, cells were treated with 10 μM probe for 25 min. Then these cells were thoroughly washed thrice and these pre-treated cells were further incubated with 20 μM HOCl for another 20 min prior to the imaging studies.

Colocalization studies with ER-tracker green:

Sample Name	Pearson's Coefficient (PCC)	Mandar's Coefficient		Threshold channel		Overlap Coefficient
		M1	M2	ER (500-520) nm	SF-1 (570-620) nm	
SF-1 co-stained ER-Tracker Green	0.0446	0.0999	0.0999	800	300	0.822

Colocalization Experiment with Cytopainter Golgi Tracker Green:

Sample Name	Pearson's Coefficient (PCC)	Mandar's Coefficient		Threshold channel		Overlap Coefficient
		M1	M2	Mito (500-520) nm	SF-1 (570-620) nm	
SF-1 co-stained Cytopainter Golgi Green	0.98	0.981	0.97	487	241	0.9

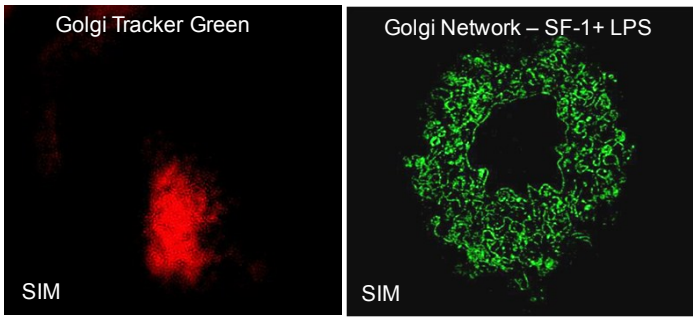

Figure S12. Comparison SIM images: Golgi Tracker Green and **SF-1** showing superior stability of **SF-1** over Golgi Tracker Green.

The superior stability of **SF-1** probe could be seen in the Fig. S12. SF-1 probe excellent SIM image sustaining the harsh SIM conditions when compared to the conventional dye namely Golgi Tracker available commercially. This shows the excellent suitability of SF-1 as a Structured Illumination Microscopy (SIM) probe.

Structured Illumination Microscopy Experiments of SF-1 probe

(A) General Description:

Structured Illumination diffracts the beam in to three parallel beams and they are combined by the objective to produce 3D interference fringe patterns in the sample. Multiple images are obtained by adjusting the fringe pattern and by slicing through the sample with respect to different focal planes generating a series of images of the sample and this Image volume is known as Z-stack. Each frame of the Z-stack is reconstructed so that it could provide definitive information of the details of the sample which we are Imaging thereby improving the resolution close to two fold which is not achieved by using conventional light microscopy.

(B) Sample preparation (SIM and Wide Field Microscopy):

RAW 264.7 cells were seeded on cover slips (22 mm X 22 mm, 170 ± 5 μm square Cover glasses) placed in six well plates in DMEM culture medium containing (10% FBS and 1% Penicillin Streptomycin) for 24 hours at 37°C, 4% CO_2. After 24 hours when 70% confluency was achieved the cells were washed with DMEM culture medium then cells were treated with **SF-1** (10 μM) for 25 minutes. Cells were then washed thrice with culture medium and further treated with different NaOCl for 20 minutes. After that cells were washed again with Phosphate Buffer Saline (2X PBS). After carrying out the Live cell uptake of the **SF-1** probe and the small molecule, the cells were fixed with 4% PFA for 15 minutes and then washed thrice with PBS and two times and then the cover slips were mounted using mounting medium (Vectashield h-1000).The coverslips were then sealed using nail varnish and the sample were then imaged. As Structured Illumination Microscopy (SIM) relies on the cell morphology, the cells were examined with light microscope and then imaged using SIM.

Structured Illumination Microscopy and High Resolution Microscopy:

Instrument Specification:

The Delta Vision OMX system is a Microscope which surpasses normal microscopy resolution limits. It allows to image beyond the surface of the coverslips by using multiple probes to retrieve exhaustive biological information from all directions. This Instrument's Structured Illumination Microscopy technology enables to image deeply the biology and

resolves features which are literally close to invisible through traditional light microscopy. Delta Vision OMX can image from one to two microns in to cells and tissues. Delta Vision OMX is a very flexible microscope and it works well with all kinds of probes including conventional fluorophores to artificially engineering fluorochromes.

SIM and Wide Field Microscopy Data Acquisition and Data Processing:

(A) Structured Illumination Microscopy (SIM):

Structured Illumination Microscopy (SIM) carried out by us using the Delta Vision OMX is basically a Wide Field Microscopy technique based on Moire's effect. The Resolution improvement is achieved based on the Reconstruction of the acquired image by using the inbuilt software namely Soft Worx. The Z stacks acquired during the Imaging are post processed by using the reconstruction option of Soft Worx. SIM acquisition is dependent mainly on the imaging parameters and acquisition parameters and this varies depending on the sample and in particularly on the nature of the probe. The **SF-1** probe was Excited at 568 nm and the emission was collected at 586 nm (Alexa Flour 568 Channel of the Delta Vision OMX). In the case of **SF-1** probe the Structured Illumination experimental condition employed for running the SI experiment for single colour experiments were mainly dependent on the thickness of the Z stack (sections 80 to 100), section spacing (0.125 to 0.150), thickness of the sample (8 to 10). As **SF-1** probe is a very bright probe the SIM acquisition, we needed to vary the %T and exposure time. Therefore in all our Single colour experiments the exposure time was between 3 to 10 and the %T was in the range of 10 to 50. The Colocalization experiments were performed with both ER Tracker Green and Cytopainter Golgi Green. The Dual colour experiments were performed with Hoechst. In both of these experiments the SIM conditions of these Co-staining agents were maintained in accordance to the **SF-1** probe which would be discussed in the later sections.

(B) Wide Field Fluorescence Microscopy (WF):

The Wide Field Fluorescence Microscopy involves collection of greater quantity of light (Including out of focus light) compared the confocal microscopy technique which involves loss of more than 30% of light as out of focus light is discarded during image acquisition as it being a Pointilistic technique. The Wide field Microscopy technique involves improvement in resolution only after post processing the Z-stacks acquired. Post processing of acquired data

is done by using Soft Worx software provided in the Delta Vision OMX. Off line image processing for the manuscript was carried out by using Fiji software. The **SF-1** probe was excited at 568 nm and the emission was collected at 586 nm (Alexa Flour 568 Channel of the Delta Vision OMX). The Wide Field imaging conditions employed for Single Colour imaging of the RAW cells by using the **SF-1** probe are, thickness of the Z stack (sections 40 to 100), section spacing (0.250 to 0.500), thickness of the sample (8 to 15). As **SF-1** probe is a very bright probe the SIM acquisition we need to vary the %T and exposure time and in all our Single colour experiments the exposure time was between 50 to 100 and the %T was in the range of 30 to 50. The colocalization experiments were performed with both ER Tracker Green and Cytopainter Golgi Tracker Green. The Dual colour experiments were performed with Hoechst. In both of these experiments the SIM conditions of these Co-staining agents were maintained in accordance to the **SF-1** probe which would be discussed in the later sections. The wide field images obtained in all of the above mentioned experiments were deconvolved as mentioned in the following sections.

(C) 3D SIM Projection:

The off line processing of Structured Illumination Microscopy (SIM) images was carried out by using FIJI software. The option Stacks (3D project) was employed in obtaining a 3D projection of the Structured Illumination Microscopy images obtained by using the Delta Vision OMX-SIM Microscope. The 3D projections were also obtained by using the IMARIS 3D Software.

(D) Deconvolved Wide Field Microscopy:

The Deconvolution procedure involves the processing of the raw Wide Field images obtained from the Delta vision OMX. This Image processing is carried out by using the Soft Worx software which is used for carrying out the post processing of the SIM data. Deconvolution is a computationally intensive image processing technique which helps in improving the contrast and Axial resolution post acquisition of the images from 400 nm to 350 nm. During the Deconvolution procedure the raw Wide Field images are processed by removing the out of focus blur from stack of acquired images called Z-Stack. The Deconvolution in effect improves the Quasi random disarrangement, improves the signal to noise ratio, improves the quality of the point spread function and thereby retrieves more information from the post processed wide field image and hence contributes to an improved resolution.

Intracellular HOCl Detection Experiments (SIM and Wide Field Microscopy):

(A) Detection by External Addition of HOCl:

The experiment was carried out by incubating the 70% confluent RAW cells with 10 μM **SF-1** in DMEM medium with 10% FBS and 1% Penicillin Streptomycin at 37°C, 5% CO_2 for 25 minutes. The cells were further treated with varied concentration of NaOCl as mentioned above for 30 minutes. The increase in *Incellular* luminescence with increase of HOCl concentration in the cells was imaged by Structured Illumination Microscopy (SIM) and also Wide Field Fluorescence Microscopy. Both live and fixed cell imaging were carried out by using Wide Field Fluorescence Microscopy. SIM was carried out only on fixed cells.

(B) Endogenous Detection of HOCl:

In order to determine whether the **SF-1** probe could detect HOCl generated inside the cells, we carried out an experiment with lipopolysaccharide (LPS) which is known to stimulate the generation of HOCl and other reactive oxygen species inside the cells. The aim was to find out if there is an increase in *Incellulo* luminescence with increase in concentration of LPS and image this using Structured Illumination Microscopy. The experiment was carried out by incubating the 70% Confluent RAW cells with increase in concentration of LPS from 100 ng to 2500 ng in DMEM medium with 10% FBS and 1% Penicillin Streptomycin at 37°C, 5% CO_2 for 24 hours. The cells were further treated with 10 μM of **SF-1** probe for 30 minutes. The increase in *incellular* luminescence with increasing concentration of LPS in the cells was imaged by Structured Illumination Microscopy and also Wide Field Fluorescence Microscopy.

(C) Intracellular Signal attenuation and spreading experiment:

A time lapse LPS experiment was required to determine the origin of signal development after the formation of SF-2 in vivo. The aim was to determine the development and spread of emission signal proving the effectiveness of the probe in detecting specific signals developing from specific intracellular organelle. This experiment was carried out on fixed cells using Structured Illumination Microscopy. The experiment was carried out by incubating the 70% confluent RAW cells in DMEM medium with 10% FBS and 1% Penicillin Streptomycin at 37°C, 5% CO_2 for 24 hours. The cells were first treated with 10 μM of **SF-1** probe for 30 minutes and then treated with LPS (2500 ng) for specific time intervals namely 2 minutes, 15

minutes, 30 minutes, 1 hour, 2 hour, 7 hour and 2 hours. The changes in *incellular* luminescence according to different time intervals were imaged by Structured Illumination Microscopy and also Wide Field Fluorescence Microscopy.

(D) Residual HOCl signal detection – Selective HOCl detection by SF-1:

A separate experiment was carried out in parallel with the time lapse LPS experiment. Here The experiment was carried out by incubating the 70% Confluent RAW cells within DMEM medium with 10% FBS and 1% Penicillin Streptomycin at 37°C, 5% CO_2 for 24 hours. The cells were first treated with 10 μM of **SF-1** probe for 30 minutes and then treated with LPS (2500 ng) for 30 minutes and the LPS is removed from the cellular environment and replaced by growth media. The cells were then imaged using SIM. The changes in cellular luminescence were imaged by Structured Illumination Microscopy and also Wide Field Fluorescence Microscopy. This was carried out in order to determine if **SF-1** could detect any residual HOCl generated even after LPS was removed. This experiment was carried out to prove the specific HOCl detection capability of the **SF-1** probe. **SF-1** was able to detect the residual HOCl in Macrophages. After 48 hours still some signals have been recorded.

Colocalization SIM and Wide Field Microscopy Experiments:

(A) Colocalization Experiments with ER Tracker Green:

The Co-staining experiments with ER Tracker Green was carried out by Incubating the ER Tracker Green (1 μM) further for 30 minutes after incubating the RAW cells with **SF-1** probe (10 μM) initially for 25 minutes and HOCl (20 μM) for a further 20 minutes. The Cells were washed regularly three times with DMEM culture media and PBS. The Cellular uptake of both the probes are carried out in live cells and then the cells were fixed with 4% PFA and mounted and navigated initially for proper cell morphology by using Light Microscope and then imaged by using Structured Illumination Microscopy (SIM) and Wide Field Microscopy (WF). The **SF-1** probe was excited at 568 nm and the emission was collected in the Alexa Fluor Channel (570 nm to 620 nm) and the ER-Tracker Green was excited at 488 nm and the Emission was collected in the **FITC** Channel (500 nm to 550 nm). The SIM imaging conditions maintained are, For **SF-1** probe: Thickness of the Z stack (sections 50 to 100), section spacing (0.125 to 0.150), Thickness of the sample (8 to 11), Exposure time was between 3 to 30 and the %T was in the range of 10 to 50. For **ER Tracker Green**: Thickness

of the Z stack (sections 50 to100), section spacing (0.125 to 0.150), thickness of the sample (8 to 11), exposure time was between 10 to 50 and the %T was in the range of 10 to 50. The WF imaging conditions maintained are, For **SF-1** probe: Thickness of the Z stack (Sections 40 to 80), Section spacing (0.250 to 0.500), thickness of the sample (8 to 11), exposure time was between 1 to 30 and the %T was in the range of 2 to 30. For **ER Traker Green**: thickness of the Z stack (sections 40 to 80), section spacing (0.250 to 0.500), thickness of the sample (8 to 11), exposure time was between 10 to 50 and the %T was in the range of 2 to 30.

(B) Colocalization Experiments with Cytopainter Golgi Tracker Green:

The Co-staining experiments with Cytopainter Golgi Tracker Green was carried out by Incubating the Cytopainter Golgi Tracker Green (1 μM) further for 30 minutes after incubating the RAW cells with **SF-1** probe (10 μM) initially for 25 minutes and HOCl (20 μM) for a further 20 minutes. The cells were washed regularly three times with DMEM culture media and PBS. The cellular uptake of both the probes are carried out in live cells and then the cells were fixed with 4% PFA and mounted and navigated initially for proper cell morphology by using Light Microscope and then imaged by using Structured Illumination Microscopy (SIM) and Wide Field Microscopy (WF). The **SF-1** probe was excited at 568 nm and the emission was collected in the **Alexa Fluor** Channel (570 nm to 620 nm) and the Cytopainter Golgi Tracker Green was excited at 488 nm and the emission was collected in the **FITC** Channel (500 nm to 550 nm). The SIM imaging conditions maintained are, For **SF-1** probe: thickness of the Z stack (sections 50 to 100), section spacing (0.125 to 0.150), thickness of the sample (8 to 11), exposure time was between 10 to 50 and the %T was in the range of 2 to 30. The SIM imaging conditions maintained are, For **Golgi Tracker Green**: Thickness of the Z stack (Sections 50 to 100), Section spacing (0.125 to 0.150), thickness of the sample (8 to 11), exposure time was between 10 to 50 and the %T was in the range of 2 to30. The WF imaging conditions maintained are, For **SF-1** probe: Thickness of the Z stack (Sections 30 to 60), section spacing (0.250 to 0.500), thickness of the sample (8 to 11), exposure time was between 2 to 30 and the %T was in the range of 2 to 30. For **Golgi Tracker Green:** Thickness of the Z stack (Sections 80 to 100), Section spacing (0.250 to 0.500), Thickness of the sample (8 to 11), Exposure time was between 2 to 30 and the %T was in the range of 2 to 30.

(C) Colocalization Experiments with Lyso Tracker Deep Red:

The Co-staining experiments with Lyso Tracker Deep Red was carried out by incubating the Lyso Tracker Deep Red (500 nM) further for 30 minutes after incubating the RAW cells with **SF-1** probe (10 μM) initially for 25 minutes and HOCl (20 μM) for a further 20 minutes. The Cells were washed regularly three times with DMEM culture media and PBS. The Cellular uptake of both the probes are carried out in live cells and then the Cells were fixed with 4% PFA and mounted and navigated initially for proper cell morphology by using Light Microscope and then imaged by using Structured Illumination Microscopy (SIM) and Wide Field Microscopy (WF). The **SF-1** probe was excited at 568 nm and the emission was collected in the **Alexa Fluor** Channel (570 nm to 620 nm) and the Lyso Tracker Deep Red was excited at 644 nm and the emission was collected in the Cy5 Channel (> 670 nm). The SIM imaging conditions maintained are, For **SF-1** probe: Thickness of the Z stack (Sections 50 to 100), section spacing (0.125 to 0.150), Thickness of the sample (8 to 11), Exposure time was between 10 to 50 and the %T was in the range of 2 to 30. **For Lyso Tracker Deep Red**: Thickness of the Z stack (Sections 50 to 100), Section spacing (0.125 to 0.150), Thickness of the sample (8 to 11), Exposure time was between 10 to 50 and the %T was in the range of 2 to 30. The WF imaging conditions maintained are, For **SF-1** probe: Thickness of the Z stack (Sections 30 to 60), section spacing (0.250 to 0.500), thickness of the sample (8 to 11), exposure time was between 2 to 30 and the %T was in the range of 2 to 30. For **Lyso Tracker Deep Red:** Thickness of the Z stack (Sections 80 to 100), section spacing (0.250 to 0.500), thickness of the sample (8 to 11), exposure time was between 2 to 30 and the %T was in the range of 2 to 30.

Dual Colour SIM and Wide Field Microscopy Experiments:

The Dual colour experiments with Hoechst as the nuclear stain was carried out by Incubating the Hoechst (500 nM) further for 30 minutes after incubating the RAW cells with **SF-1** probe (10 μM) initially for 25 minutes. The cells were washed regularly three times with DMEM culture media and PBS. The cellular uptake of both the probes are carried out in live cells and then the cells were fixed with 4% PFA and mounted and navigated initially for proper cell morphology by using light microscope and then imaged by using Structured Illumination Microscopy (SIM) and Wide Field Microscopy (WF). The **SF-1** probe was excited at 568 nm

and the emission was collected in the **Alexa Fluor** Channel (570 nm to 620 nm) and the Hoechst was excited at 405 nm and the emission was collected in the **DAPI** channel (420 nm to 500 nm). The SIM imaging conditions maintained are, For **SF-1** probe: Thickness of the Z stack (Sections 40 to 80), Section spacing (0.125 to 0.150), Thickness of the sample (5 to 8), Exposure time was between 10 to 50 and the %T was in the range of 10 to 50. The SIM imaging conditions maintained are, For **Hoechst**: Thickness of the Z stack (Sections 40 to 80), Section spacing (0.125 to 0.150), thickness of the sample (5 to 8), exposure time was between 50 to 100 and the %T was in the range of 10 to 50. The WF imaging conditions maintained are, For **SF-1** probe: thickness of the Z stack (Sections 20 to 50), section spacing (0.250 to 0.500), thickness of the sample (5 to 9), exposure time was between 10 to 50 and the %T was in the range of 2 to 30. The WF imaging conditions maintained are, For **Hoechst**: Thickness of the Z stack (Sections 20 to 50), Section spacing (0.250 to 0.500), thickness of the sample (5 to 9), Exposure time was between 10 to 50 and the %T was in the range of 2 to 30.

Dual Colour SIM image:

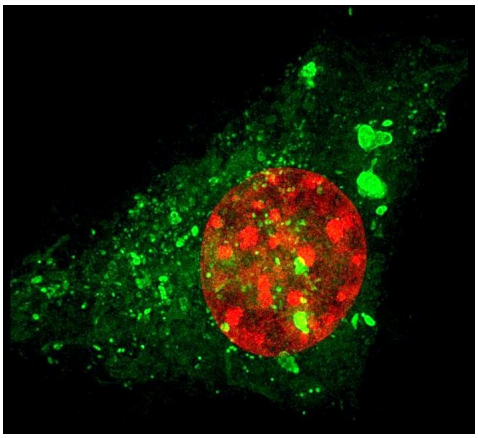

Figure S13. Dual 3D-SIM using **SF-1** pre-treated with LPS and Hoechst 33442 (psudo green colour was used for probe **SF-1** and psudo red colour is for nuclear staining agent Hoechst).

HRMS of SF-2:

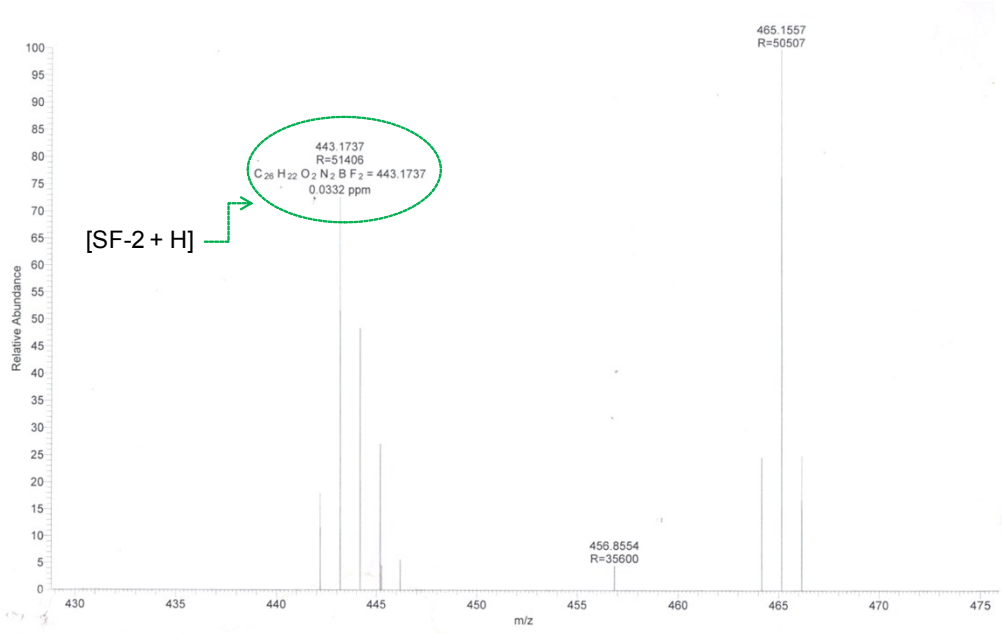

Figure S 14. HRMS of **SF-1** in presence of HOCl.

Co-localization studies with SF-2 with Golgi tracker and Lyso tracker:

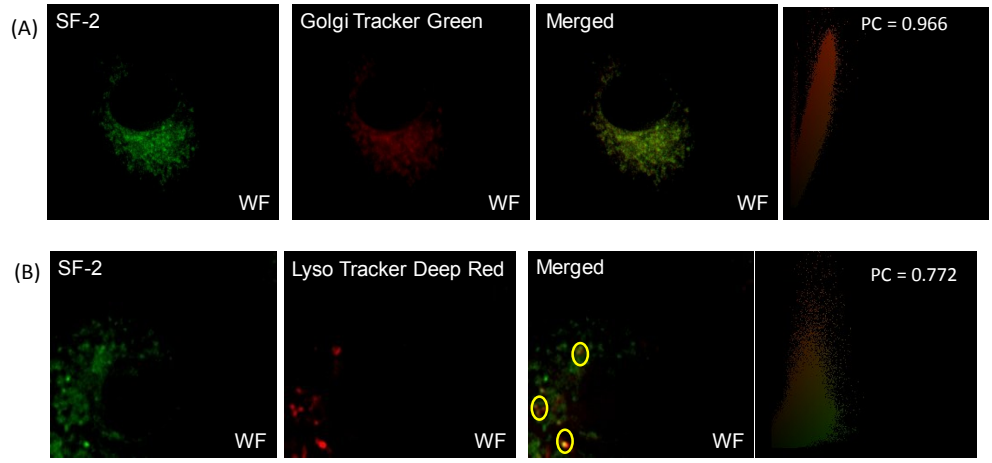

Figure S 15. Colocaliztion studies of SF-2 with (A) Golgi tracker green; (B) Lyso tracker deep red.

MTT Assay of SF-2:

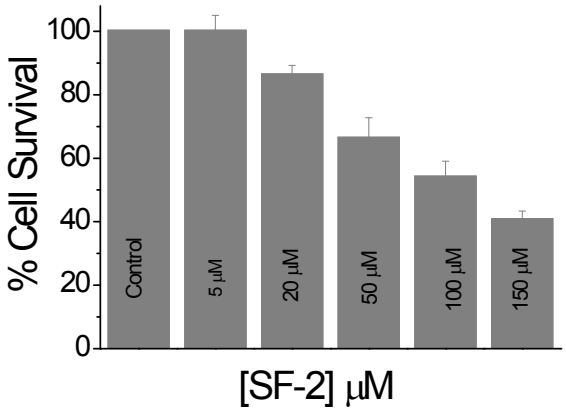

Figure S16. MTT assay to determine the cell viability percentage in presence of **SF-2** in RAW 264.7 cells. IC_{50} was found to be 110 μM.