

Phenylenevinylene Conjugated Oligoelectrolytes as Fluorescent Dyes for Mammalian Cell Imaging

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Experimental details

1. Synthesis of conjugated oligoelectrolytes.

The 1,4-bis(4'-(N,N-bis(6'' (N,N,N trimethylammonium)hexyl)amino)-styryl) benzene tetraiodide (DSBN⁺) and 4,4'-bis(4'-(N,N-bis(6''-(N,N,N-trimethylammonium)hexyl)amino)-styryl) stilbene tetraiodide (DSSN⁺), were prepared as previously described [L. E. Garner, J. Park, S. M. Dyar, A. Chworos, J. J. Sumner and G. C. Bazan, *J Am Chem Soc*, 2010, **132**, 10042].

The longer five-ring analog (COE1-5C) has been prepared according to literature precedent [H. Hou, X. Chen, A. W. Thomas, C. Catania, N. D. Kirchhofer, L. E. Garner, A. Han and G. C. Bazan, *Adv Mater*, 2013, **25**, 1593.].

2. Cell cultures

The human cervical carcinoma cells (HeLa) and the human chronic myeloid leukemia cells (K562) were cultured in RPMI 1640 medium (Gibco, Invitrogen), with 10% addition of FBS (Gibco, Invitrogen) and 100 U/ml penicillin and 100 µg/ml streptomycin (Invitrogen). The Human Embryonic Kidney 293 Cells (HEK293T) was maintained in Dulbecco's medium (DMEM, Sigma) supplemented with 10% FBS and antibiotics (100U/ml penicillin and 100µg/ml streptomycin). All cell lines used in our experiments were grown at 37 °C under a 5% CO₂ atmosphere.

3. Cytotoxicity assay

Cytotoxicity of the conjugated oligoelectrolytes was assessed through a series of experiments on human cell lines, both cancerous and non-cancerous. Cell viability was quantified using standard MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) (Sigma). The principle of this rapid screening test relies on the conversion of the tetrazolium salt into purple formazan by mitochondrial dehydrogenase, an enzyme active only in living cells. The MTT assay, which allows precise quantification of cell viability, was also cross-checked via cell counting by eye using an optical microscope.

The cells were trypsinized (0,05% trypsin, Gibco), diluted with proper culture medium and seeded on 96-well plate at a density of 5x10³ cells per well. After 24 hours of incubation at

37°C in an atmosphere of 5% CO₂, the COEs were added to each well to a final concentration of 1µM, 10µM or 20µM. The cells were incubated for 48 or 72 hours prior to cytotoxicity assay. Once the allotted incubation time was complete 25µl of MTT solution (5mg/ml) was added and cells were incubated for 2 hours at 37 °C in 5% CO₂ atmosphere. Finally, 95µl of lysis buffer (20% SDS, 50% aqueous dimethylformamide, pH 4.5) was added to each well and cells were incubated overnight. The amount of formazan formed was measured using a Synergy HT plate reader (BIO-TEK) at 570nm with 630nm reference wavelength. The level of cell viability was estimated relatively to the control (non-treated) cell sample. Each MTT experimental data point represents the mean ± standard error from three independent measurements repeated a minimum of five times.

4. Visualization of COEs in human cells

Cells were seeded into a chambered microscope slide (Nunc) to a density of 30x10³ per well or alternatively into 96-well plate at a density of 5x10³ cells per well in the appropriate culture medium. After 24 hours of incubation at 37 °C under a 5% CO₂ atmosphere, the culture medium was replaced with RPMI and COEs were added to a final concentration of 1µM. After 24 hours, cells were washed PBS buffer (IITD) and fixed with 3,8% paraformaldehyde solution for 10min at room temperature. The cells were then washed three times with PBS and sealed with a cover slip using DABCO/glycerol mounting medium.

5. The labeling of mitochondria in cells with MitoTracker dye

To determine if COEs localize within mitochondria we used commercially available MitoTracker dye (Invitrogen/Molecular Probes). After 24 hours of incubation at 37°C in 5% CO₂ atmosphere HeLa cells in RPMI media containing 1µM COEs were treated with MitoTracker added to a final concentration 100nM. The mitochondria were stained under these conditions for 1 hour. Then cells were washed with PBS, fixed using 3,8% paraformaldehyde for 10min at room temperature and then washed three times with PBS. In order to facilitate nucleus visualization, samples were additionally stained with DAPI (Sigma-Aldrich). Finally, the slides were prepared using DABCO/glycerol mounting medium as described above.

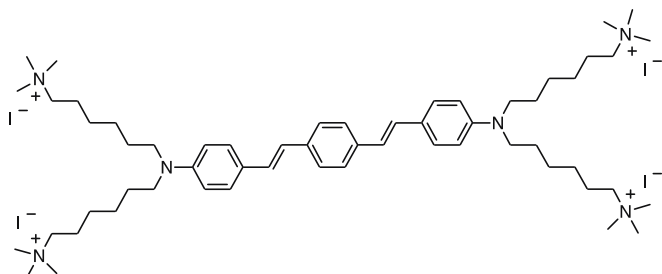
6. DAPI staining assay

The HeLa cells were incubated in RPMI medium supplemented with COEs at a concentration of 1µM for 24 hours. After incubation cells were washed with PBS and fixed with 3,8% paraformaldehyde for 10min at room temperature and again washed with PBS. Staining was performed by incubation of cells in the dark for 10min following treatment with 5µg/mL DAPI (Sigma-Aldrich). After staining, the cells were washed again three times with PBS and covered with slip using DABCO/glycerol mounting medium.

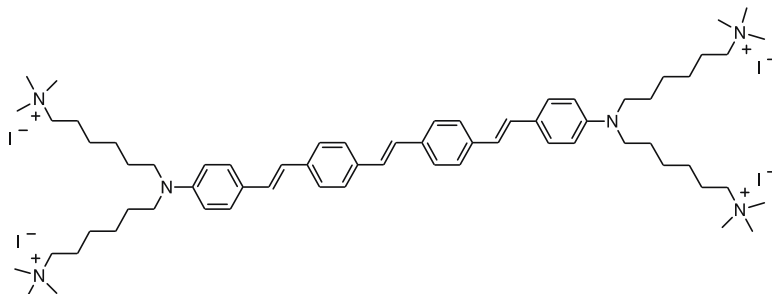
7. Golgi apparatus and Endoplasmic Reticulum staining assay

The HeLa cells were incubated in RPMI (with 10% FBS medium) and DSSN+ (1µM final) for 2 or 24 hours. Then cells were incubated with commercial dyes (BODIPY® TR Ceramide complexed to BSA or ER-Tracker Red at 2µM final) for 2 hours. After incubation cells were fixed using 3,8% paraformaldehyde for 10min and washed with PBS.

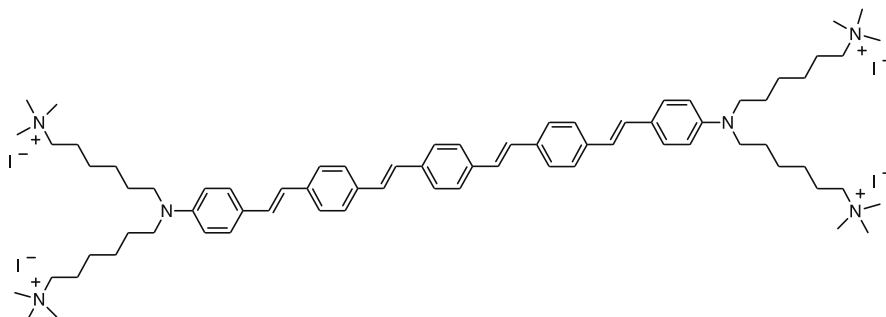
Fluorescence microscopy experiments were performed using fluorescent microscope (Nikon Eclipse series) with appropriate optical filters. The conjugated oligoelectrolytes were visualized with FITC ($\lambda_{\text{ex}} = 465 - 495$, $\lambda_{\text{DM}} = 505$, $\lambda_{\text{BA}} = 515 - 555$) and UV2A filter (long-pass types, $\lambda_{\text{ex}} = 330 - 380$, $\lambda_{\text{DM}} = 400$, $\lambda_{\text{BA}} = 420$), DAPI was visualized with a DAPI filter ($\lambda_{\text{ex}} = 340 - 380$, $\lambda_{\text{DM}} = 400$, $\lambda_{\text{BA}} = 435 - 485$) and MitoTracker was visualized using a G-2A filter ($\lambda_{\text{ex}} = 510 - 560$, $\lambda_{\text{DM}} = 575$, $\lambda_{\text{BA}} = 590$) where DM is dichroic mirror and BA is an absorption filter. Image superposition was achieved using NisElement and ImageJ software.



DSBN⁺: 1,4-bis(4'-(N,N-bis(6''-(N,N,N-trimethylammonium)hexyl)amino)-styryl) benzene tetraiodide



DSSN⁺: 4,4'-bis(4'-(N,N-bis(6''-(N,N,N-trimethylammonium)hexyl)amino)-styryl) stilbene tetraiodide



COE1-5C: 4,4'-bis(4'-(N,N-bis(6''-(N,N,N-trimethylammonium)hexyl)amino)-styryl) 1,4-distyrylbenzene tetraiodide

Figure S1. Full Chemical structures for COEs used in these studies.

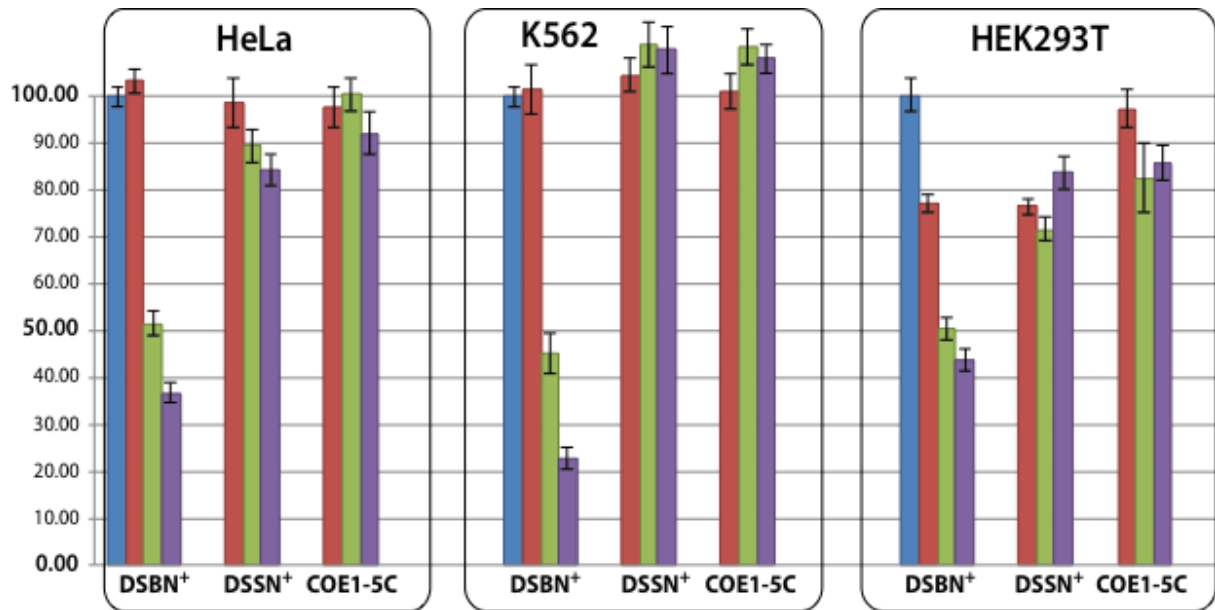


Figure S2. Viability of cells after 72 hours of incubation in the presence of various COEs: (left) adherent cancerous HeLa, (middle) suspension cancerous K562 and (right) adherent non-cancerous HEK293T. First bar (blue) represents control cell in buffer containing no COEs, following bars (red, green and violet) correspond to each of the COEs in 1, 10 and 20μM concentration, respectively.

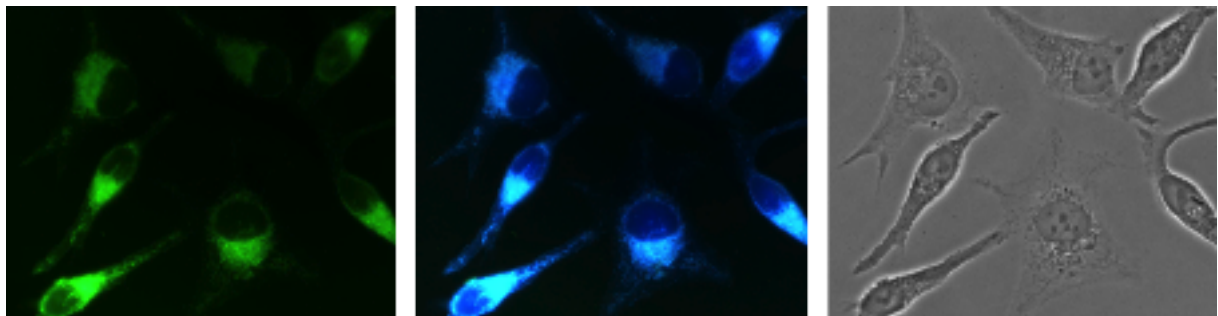


Figure S3. Fluorescence microscopy analysis of HeLa cells stained with 1μM DSSN⁺, under different wavelengths. (left) FITC filter $\square_{\text{ex}} = 465\text{-}495\text{nm}$, $\square_{\text{DM}} = 505\text{nm}$, $\square_{\text{BA}} = 515\text{-}555\text{nm}$, (center) UV2A filter $\square_{\text{ex}} = 330\text{-}380\text{nm}$, $\square_{\text{DM}} = 400\text{nm}$, $\square_{\text{BA}} = 420\text{nm}$, (right) phase contrast. DM: Dichroic mirror BA: Absorption filter.

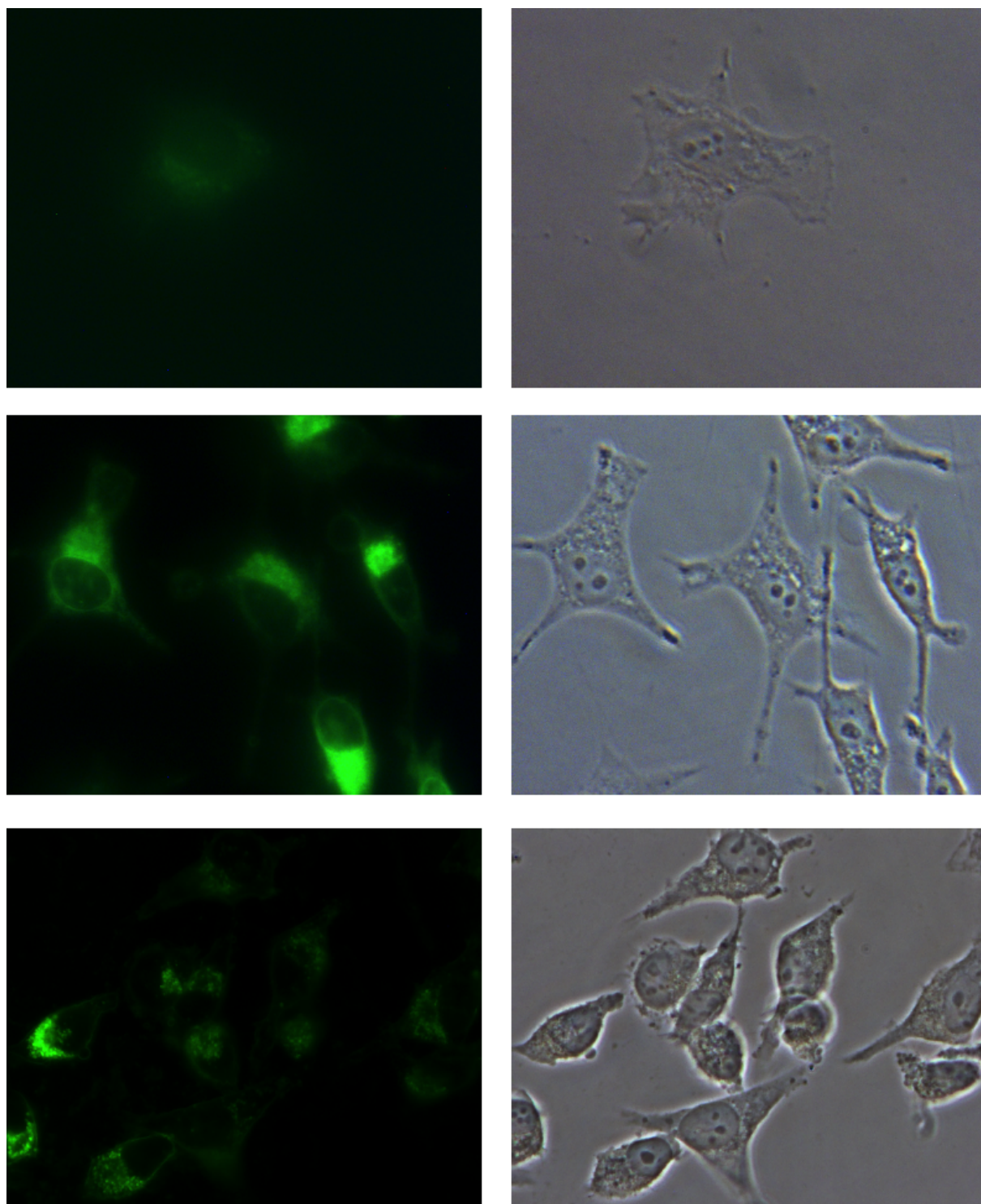


Figure S4. Fluorescent microscopy imaging of HeLa cells (fixed in formaldehyde) treated with 1mM COEs, magnification 60x, fluorescent and phase images respectively: (1st row) DSBN⁺ staining, (2nd row) DSSN⁺ and (3rd row) five member ring COE1-5C.

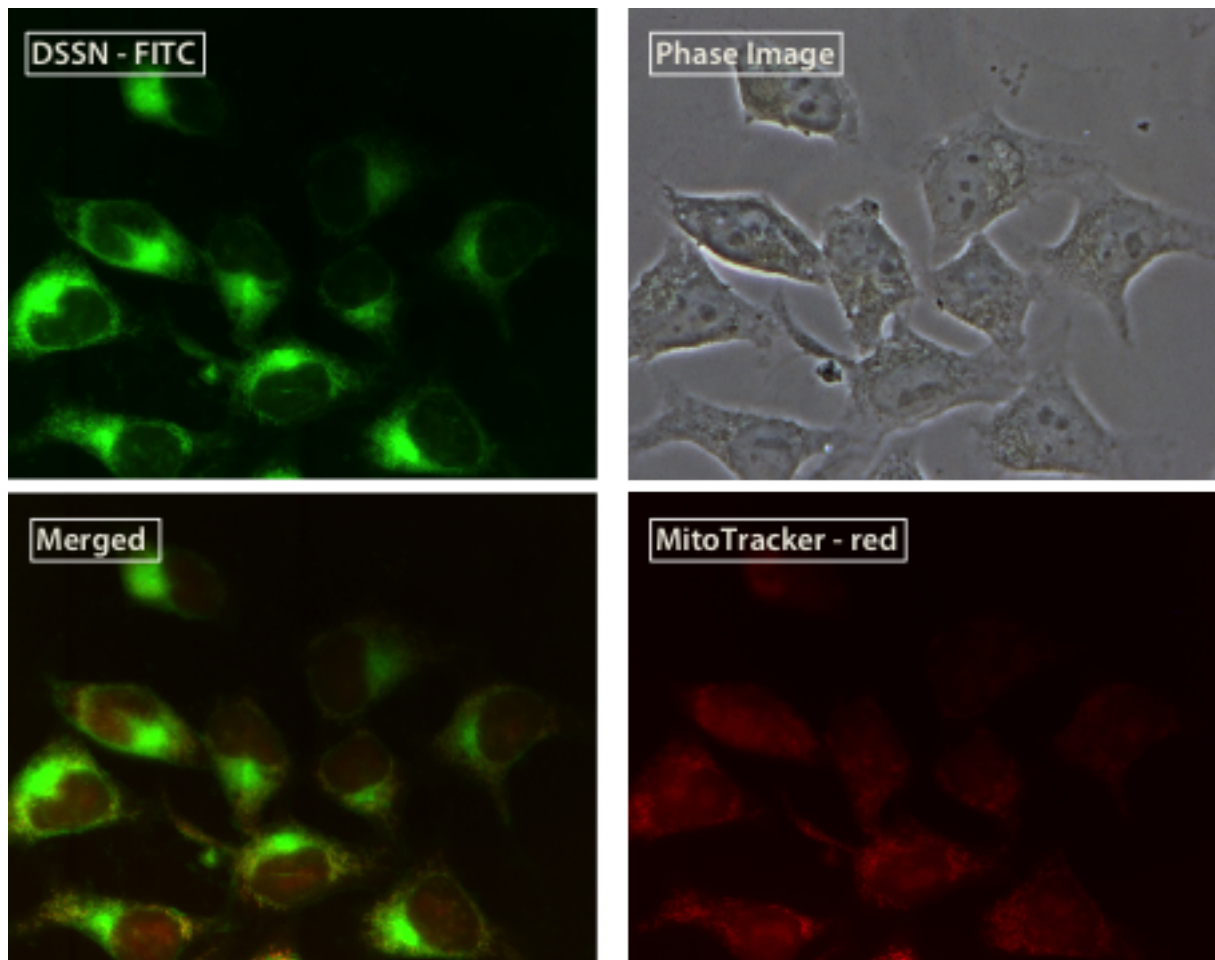


Figure S5. Fluorescent microscopy analysis showing localization of DSSN⁺ (at 1 μ M) and MitoTracker in HeLa (cells fixed) in different regions.

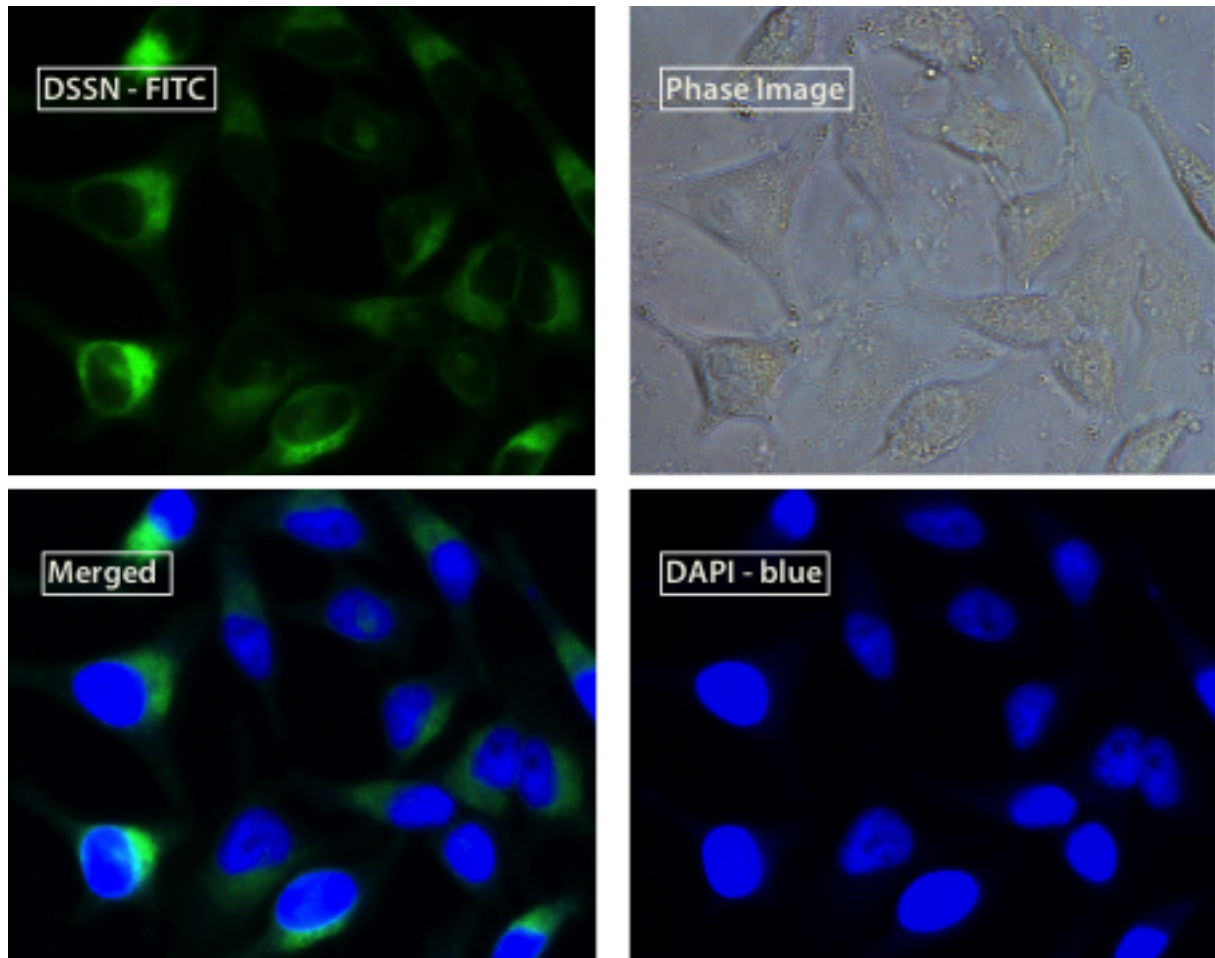


Figure S6. Fluorescent microscopy analysis showing localization of DSSN⁺ (at 1 μ M) and DAPI (cell nuclear stain) in HeLa (cells fixed) in different regions.

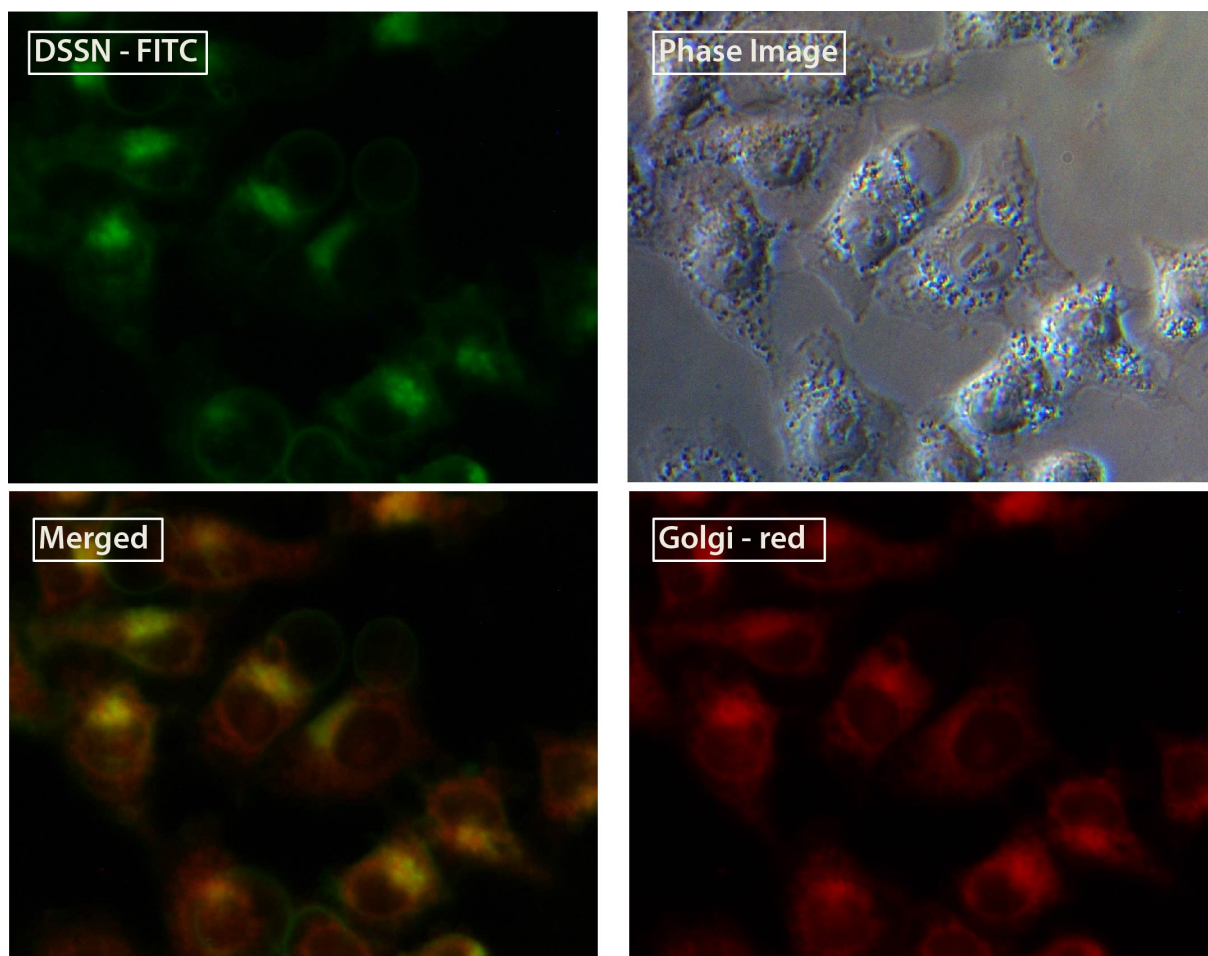


Figure S7. Fluorescent microscopy imaging for localization of DSSN⁺ (at 1 μ M) and BODIPY® TR Ceramide complexed to BSA (Golgi apparatus stain) in HeLa (cells fixed).

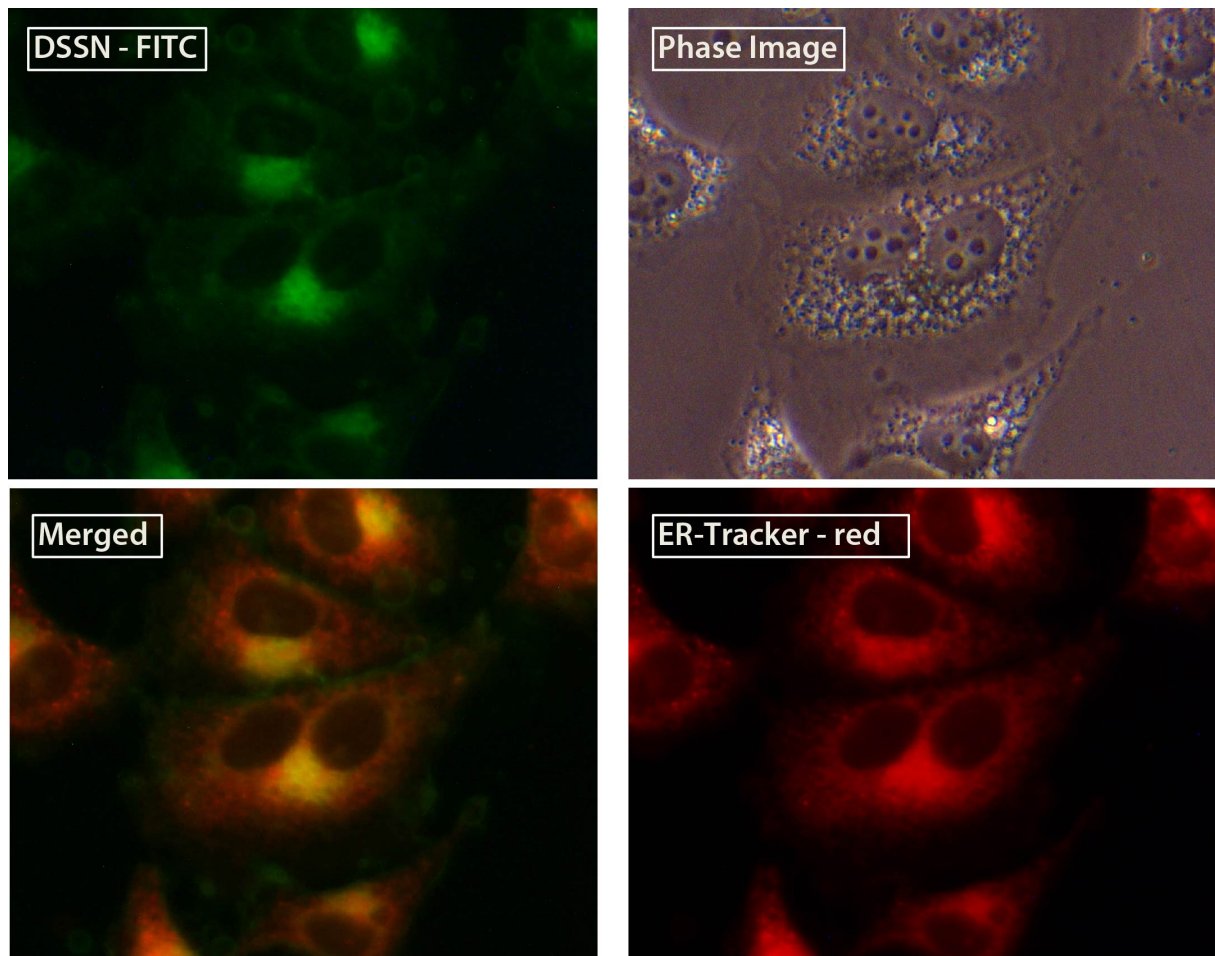


Figure S8. Fluorescent imaging of DSSN⁺ localization (at 1 μ M) with ER-Tracker Red (endoplasmic reticulum stain) in HeLa (cells fixed).