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Supplementary Information

Nonlinear optical dye TSQ1 as an efficiently selective fluorescent probe for G-quadruplex DNA

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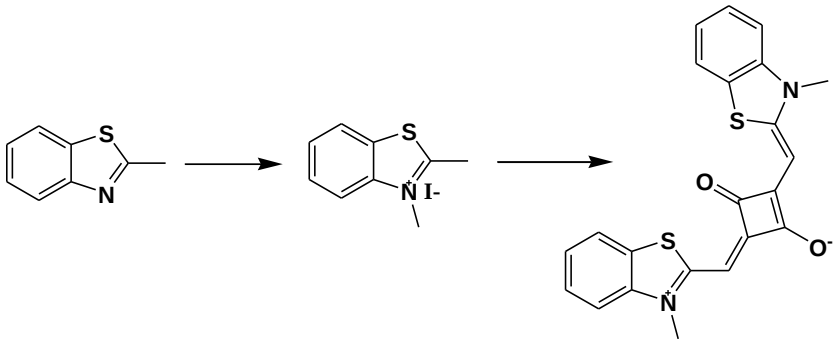
Synthesis of TSQ1



1 **2**

3-Ethyl-2-methyl-1,3-benzothiazol-3-ium Iodide (**1**). A solution of 2-methylbenzothiazole (4.48 g, 30 mmol) and iodomethane (5.60 mL, 90 mmol) in acetonitrile (250 ml) was heated under reflux for 24 h. After cooling, diethyl ether (200 ml) was added, the desired salt collected by filtration under reduced pressure and washed several times with diethyl ether. Drying under vacuum. The ether was removed. The process was repeated 1~3 times to achieve a suitable yield (5.90 g, 67.6%).

(4z)-4-[(3-methyl-6-iodo-1,3-benzothiazol-3-ium-2-yl)methylidene]-2-[(Z)-(3-methyl-6-iodo-1,3-benzothiazol-2(3H)-ylidene)methyl]-3-oxocyclobut-1-en-1-olate (TSQ1) (**2**). Condensed the quaternary ammonium salt (**1**) (0.87 g, 3 mmol) and squaric acid (0.17 g, 1.5 mmol) in BuOH/pyridine (5/1) (60 ml) at reflux for overnight. Purified from silica gel column to get blue solid (0.40 g, 33.0 %). ^{1}H NMR (CDCl3, 300 MHz) δ (ppm) 3.634 (m, 6H), 5.880 (s, 2H), 7.134-7.184 (m, 4H), 7.362 (m, 2H), 7.511-7.534 (m, 2H). HRMS (ES) calculated: 404.06532 for $C_{22}H_{16}N_2O_2S_2$; found: [M+H$^+$] 405.07510.

Fluorescent Experiments

Samples for fluorescent detection are prepared as following: The solutions (50 mM tris-HCl, pH 7.4, 50 mM NaCl) with DNA and TSQ1 compound (10μM) were maintained at 37 ℃ for about 0.5 h to achieve the equilibrium. Fluorescent spectra were detected by F900 (Combinal Steady-stata and Lifetime Spectrometer) at room temperature with excitation wavelength 686 nm, Exslit 10.0 nm, and Emslit 10.0 nm.

Circular Dichroic Studies

CD experiments utilizing a Jasco-810 spectropolarimeter (Jasco, Easton, MD, USA) were measured at room temperature using a quartz cell with a 1 cm path length; CD spectra were collected from 230 to 320 nm and with a scanning speed of 200 nm/min. The bandwidth was 5 nm, and the response time was 2 s. All CD spectra were baseline-corrected for signal contributions due to the buffer.

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Table S1 Sequences of oligonucleotides used in the present study

Name	Sequence	Description
22AG	5'-A[GGGTTA]$_3$GGG-3'	sequence from telomere DNA
G4TTA	5'-[TTAGGG]$_4$-3'	sequence from telomere DNA
h-Telo	5'-A[GGGTTA]$_3$GGGT-3'	sequence from telomere DNA
c-*myc*	5'- GAGGGTGGGGAGGGTGGGG-3'	sequence from the promoter of oncogene c-*myc*
c-kit1	5'-AGGGAGGGCGCTGGGAGGAGGG-3'	sequence from the promoter of oncogene c-kit
c-kit*	5'-GGCGAGGAGGGGCGTGGCCGGC-3'	sequence from the promoter of oncogene c-kit
H-RAS	5'-TCGGGTTGCGGGCGCAGGGCACGGGCG-3'	sequence from the promoter of oncogene RAS
c-src1	5'-GGGCGGCGGGCTGGGCGGGG-3'	sequence from the promoter of oncogene SRC
ss1	5'-CCAGTTCGTAGTAACCC-3'	complementary sequence of ss2
ss2	5'-GGGTTACTACGAACTGG-3	complementary sequence of ss1
G3T3	5'-[GGGTTT]3GGG-3'	

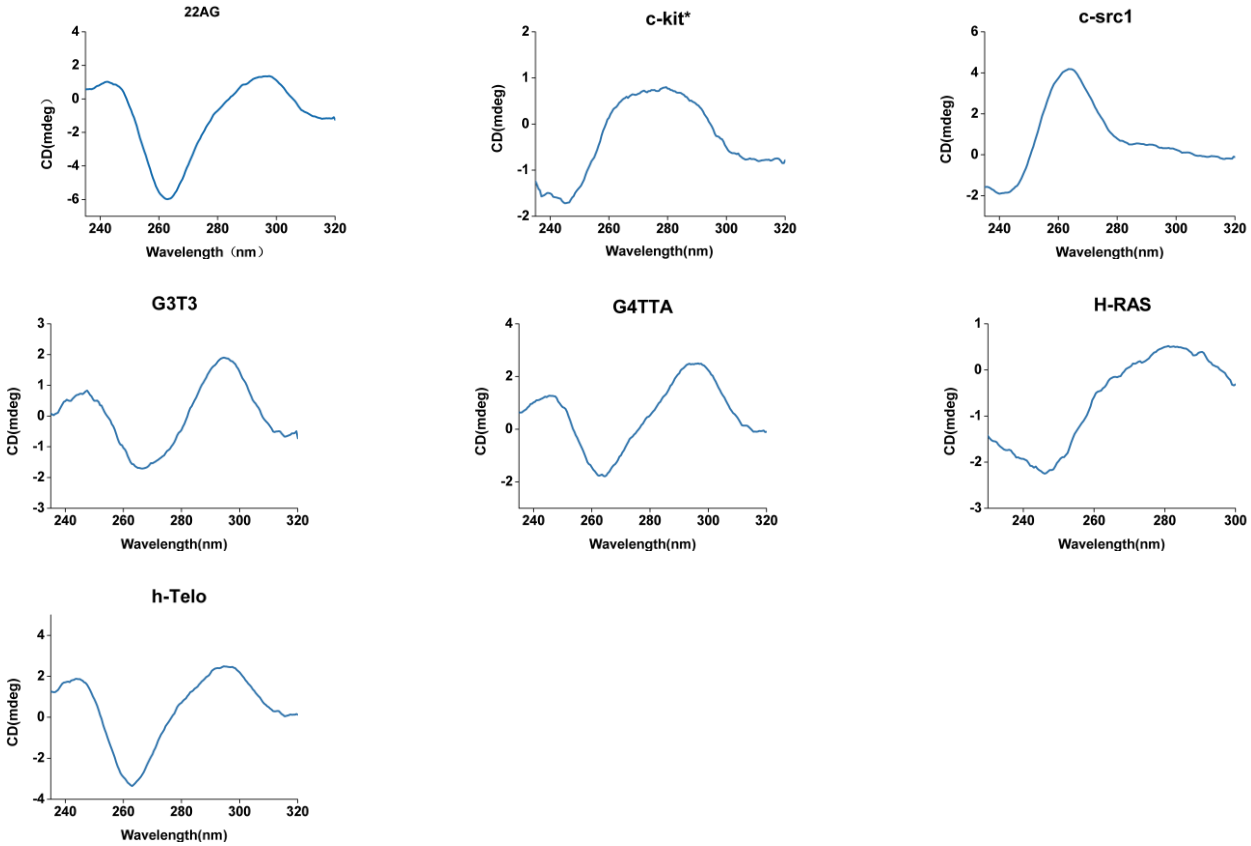

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Figure S1 CD signatures of a 10 μM solution of G-quadruplex-forming oligonucleotides in Tris-HCl buffer (50 mM, pH 7.0) containing 50 mM of NaCl at 298 K

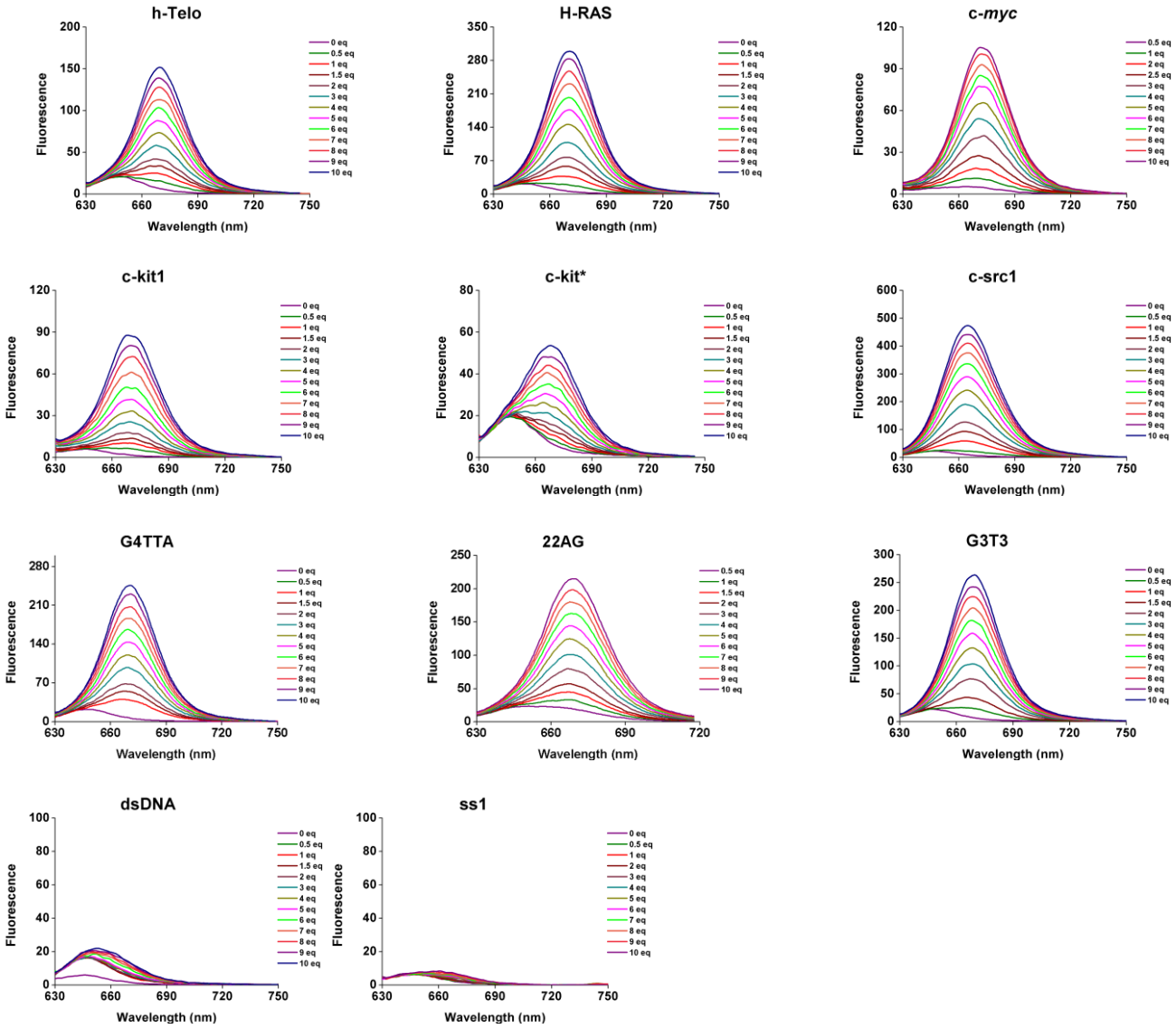

Figure S2 Fluorescence titration of a 0.5 μM solution of TSQ1 with increasing amounts of the respective oligonucleotides. Conditions: 0.5 μM TSQ1, 50 mM Tris-HCl buffer (pH 7.0), 50 mM NaCl, 298 K.

Fluorescence quantum yield

	Exciting wavelength （nm）	**Absorbance**	**Emission wavelength （nm）**	**Fluorescence intensity**
CY5	639	0.152	657	676.56
STQ1（10 μM）	620	0.043	668	92.92
STQ1（10 μM）+c-src1 （20 μM）	620	0.04	668	20.04

The fluorescence quantum yield for CY5 is 0.27.

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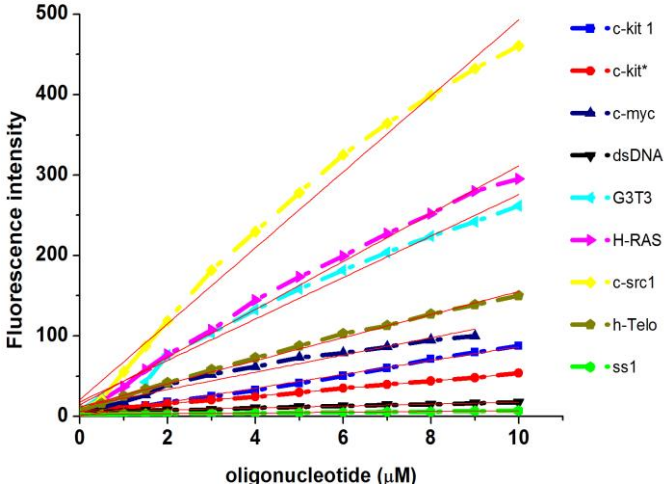
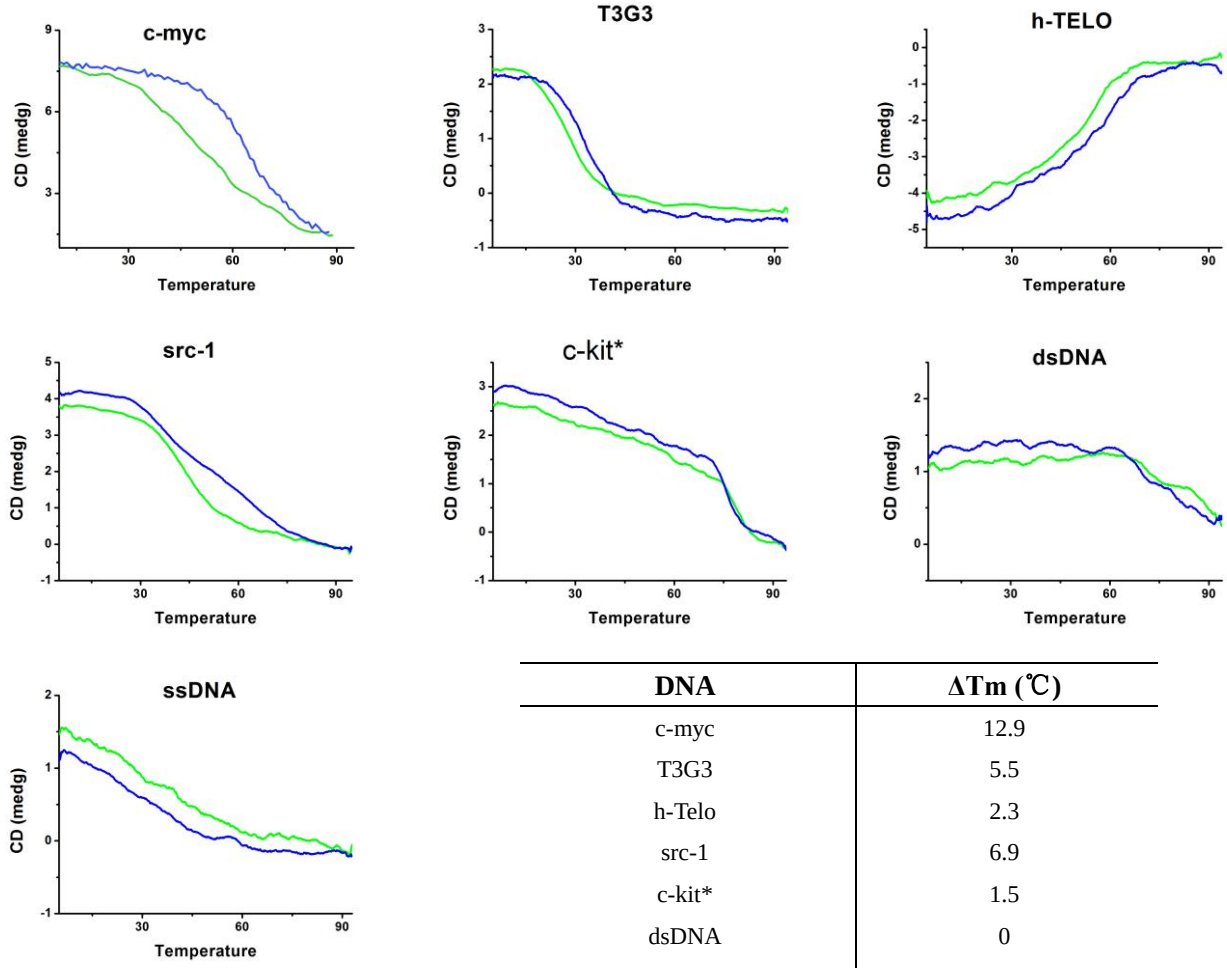


Name	Linear relationship (Adj. R-Square)
h-Telo	0.9937
c-*myc*	0.99504
c-kit1	0.99303
c-kit*	0.98597
H-RAS	0.9994
c-src1	0.9636
ss1	0.9850
dsDNA	0.9908
G3T3	0.9967

Figure S3 the linear relationship between TSQ1 and corresponding DNAs (calculate by origin).



DNA	ΔTm (℃)
c-myc	12.9
T3G3	5.5
h-Telo	2.3
src-1	6.9
c-kit*	1.5
dsDNA	0
ssDNA	0

Figure S4 FRET melting curves for experiments carried out with G-quadruplexes and ssDNA, dsDNA (10 μM) in 50 mM Tris-HCl buffer, 50 mM KCl

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with TSQ1 (100 μM). Green line: sample without TSQ1. Blue line: DNA incubate with TSQ1.

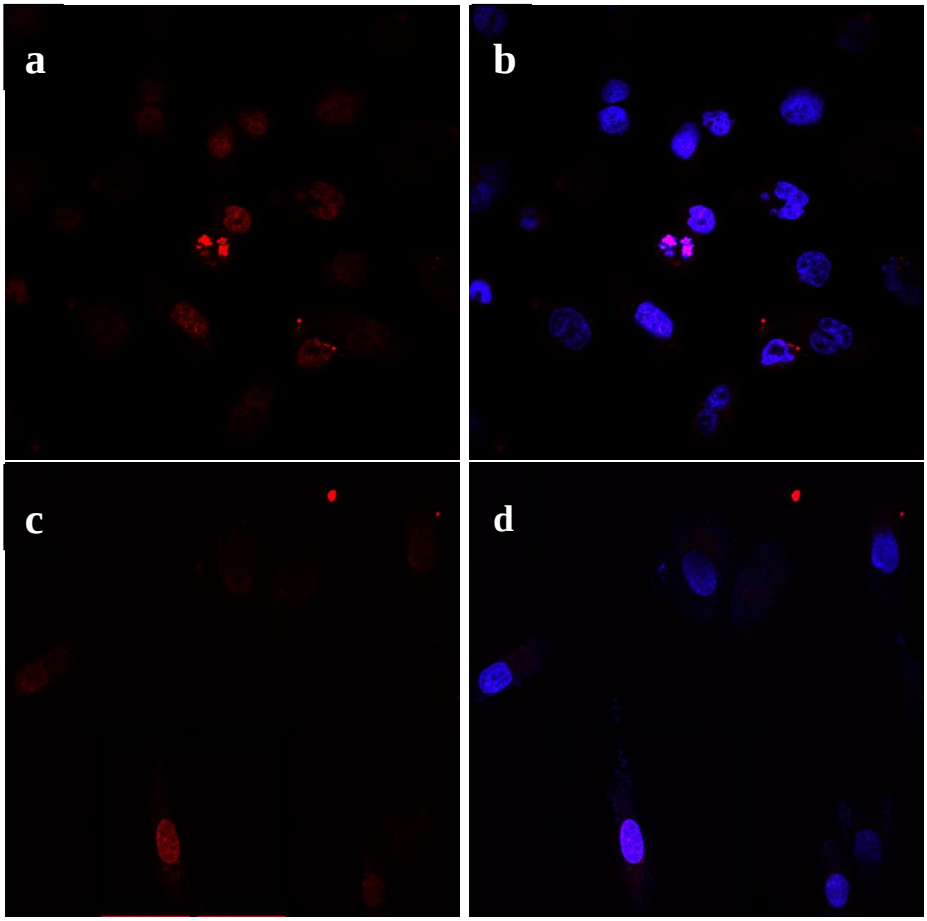

Figure S5 confocal images of CHO (a, b) and HeLa (c, d) cells cultured with TSQ1 a, c) Fluorescent image of CHO (a) and HeLa (c) incubated with TSQ1 (5μM) for 1 h at 37℃ b, d) Fluorescent image of CHO (b) and HeLa (d) incubated with TSQ1 (5μM) for 1 h at 37℃ and subsequently fixed and incubated with hoechst 33258 for 10min.

Cell culture

Human lung carcinoma cells A549, Chinese hamster ovary CHO, HeLa Cells (CCTCC, China) were cultured in MEM (Hyclone, China) supplemented with 10% FBS (Hangzhou Sijiqing Biological engineering Materials Corporation). All the cells were maintained in a humidified atmosphere of 5/95 (v/ v) CO_2/air at 37 ℃.

Confocal Laser Scanning Microscopic experiments

Human lung carcinoma cells A549, Chinese hamster ovary CHO, HeLa Cells（1×10^4 cells/well）were seeded in 35 mm cell culture glass dish. After 24 h, cells were treated with TSQ1 (5 μM) for 1 hours, then cells was washed with 3×PBS before Hoechst staining. cells were then fixed by treatment with 4% formaldehyde solution (in PBS, pH 7.4) for 15 min at room temperature and subsequently washed with PBS (pH 7.4) twice for 10 min. For colocalization experiments using Hoechst 33258, cells were treated with 5ng/μL Hoechst 33258 in 2 mL pre-warmed phenol red-free media at 37 ℃ for 30 min prior to fixing them using the abovementioned

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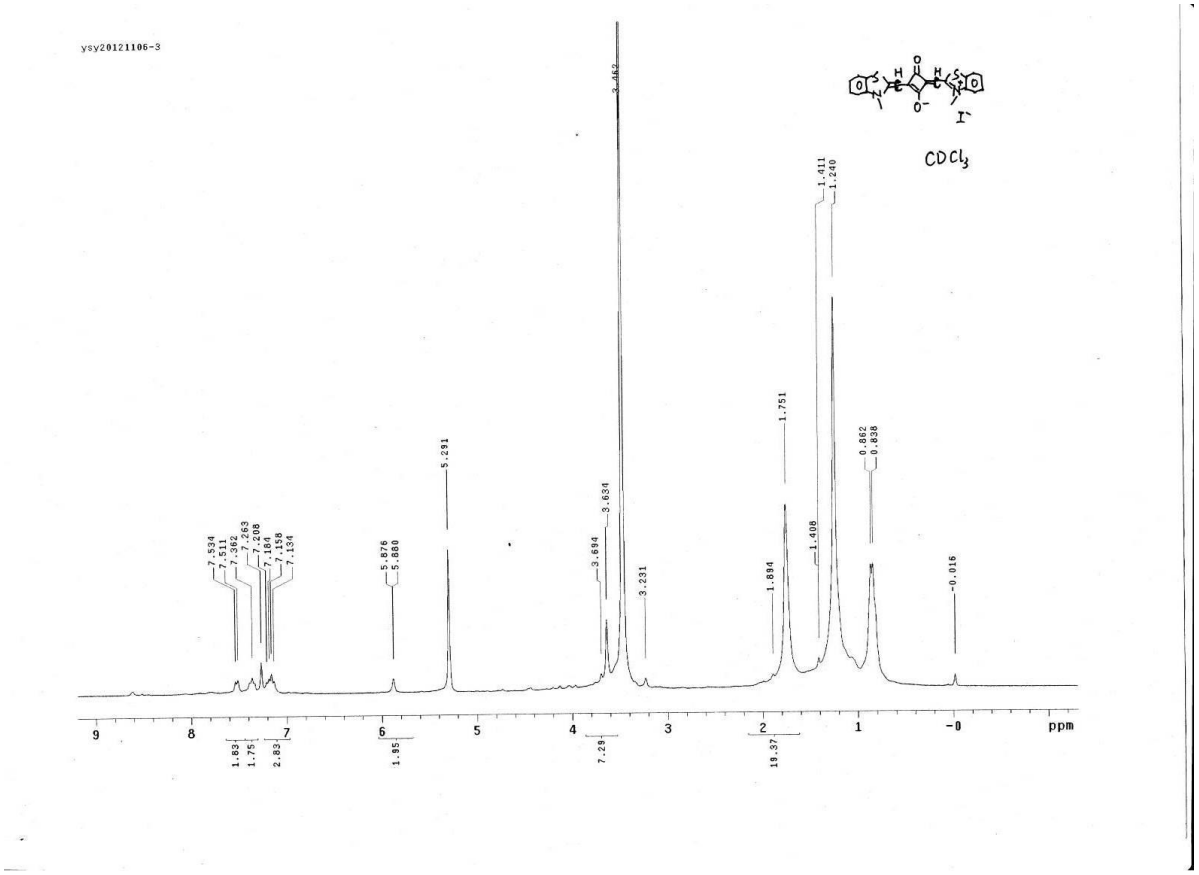
procedure. Finally, Florescent images of cells were acquired on Nikon Confocal Laser Scanning Microscope (TE2000, Japan) with an objective lens (×60). The fluorescent images were taken with green filter (excitation: 488 nm) and blue filter (excitation: 405nm). Images and merges were obtained with EZ-C1 software.

NMR for TSQ1



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HR-MS for TSQ1