Electronic Supplementary Information

Discovery of a Green DNA Probe for Live-Cell Imaging

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Contents

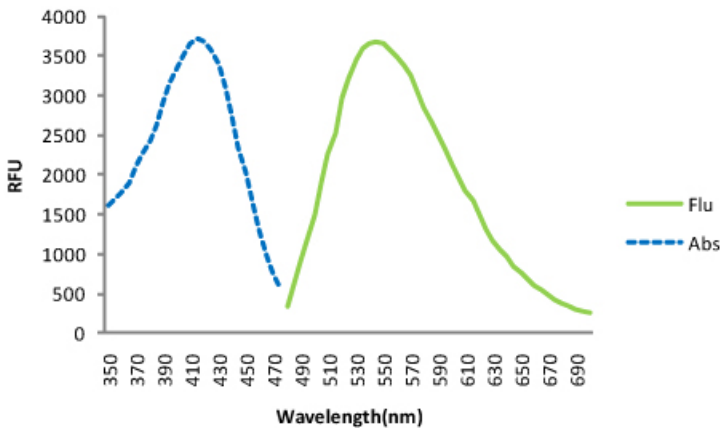


Figure S1. Absorbance and emission spectra of **C61** in ethanol (λ_{ex}= 415 nm)

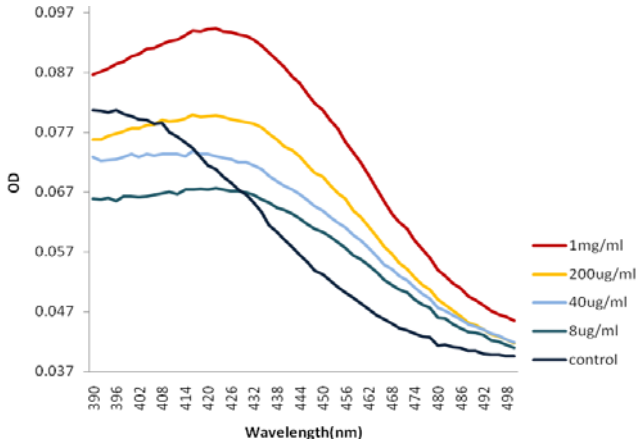


Figure S2. Photometric titration spectra for absorbance of **C61**.

DNA Sequence	K_d(μM)
$d(AATTAATT)_2$	1.9±0.2
$d(GCAAATTTGC)_2$	2.7±0.5
$d(ATCCGGAT)_2$	3.8±0.3
$d(AAAATTTT)_2$	4.0±0.5
$d(CGAATTCG)_2$	5.2±0.8
$d(CCGGCCGG)_2$	6.9±1.0
$d(CCCCGGGG)_2$	7.4±1.1

Table S1. Fluorescence binding data of C61. The dissociation constants were determined from fluorescence titrations in HEPES buffer (20mM, pH7.4).

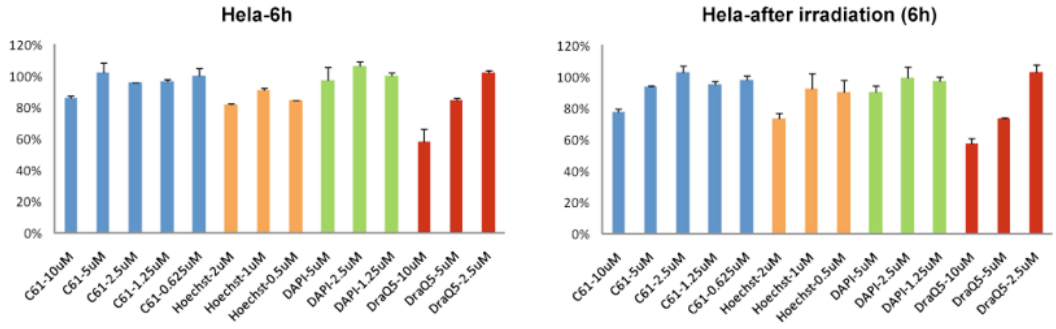


Figure S3. Cytotoxicity measurement. To compare cytotoxic effects of DNA-selective dyes, compounds were treated to cells at various concentrations covering their working concentration. After 6 hours of incubation cytotoxicity was measured by MTS assay (Promega). Blue bar represented **C61**; orange bar represented Hoechst 33342, green bar represented DAPI, and red bar represented DRAQ5. Y axis indicates percentage of cell proliferation. The GI50 of **C61** was 16.0μM.

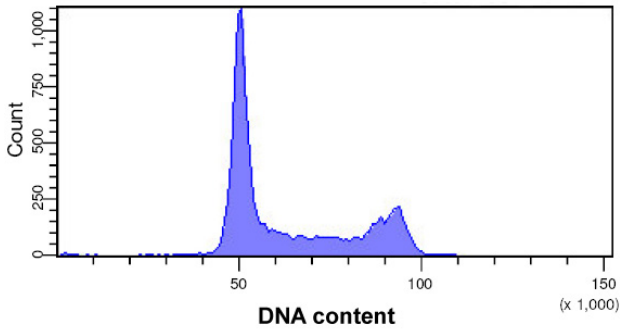


Figure S4. Cell cycle analysis using Hoechst 33342

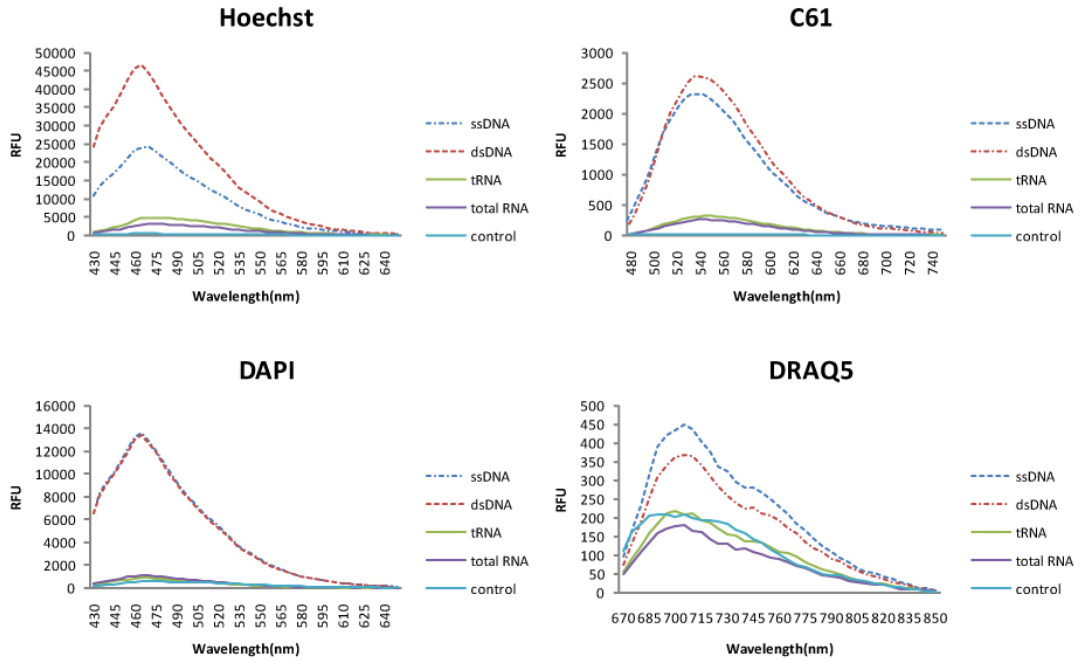


Figure S5. DNA/RNA response in vitro. For the measurement, all analytes (0.5 mg/ml) were dissolved in HEPES buffer and mixed with 10 μM of each dye. Hoechst 33342 and DAPI were excited at 350 nm and cutoff at 420 nm; **C61** was excited at 420 nm and cutoff at 475 nm; DRAQ5 was excited at 640 nm and cutoff at 665nm.

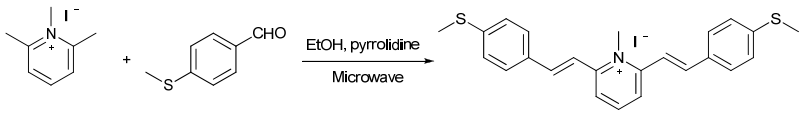
Experimental Procedure

Measurement of DNA/RNA response in vitro dsDNA from calf thymus, total RNA from torula yeast and the polynucleotides for affinity measurement were purchased from sigma and dissolved in HEPES

buffer (20mM, pH 7.4). The absorbance and fluorescence spectra of **C61** was measured by M2 microplate reader (Molecular Devices)

Cell culture and compound treatment A549 and HeLa cells were grown with RPMI medium supplemented with 10% fetal bovine serum and 100 μg/ml penicillin and streptomycin in a humidified atmosphere at 37 ºC with 5% CO_2. For cell imaging, compounds were treated to cells grown in a black optical 96-well plate for 30 min and washed. After replacements of medium, cells were subjected for imaging with ImageXpress automated acquisition (Molecular Devices). Image brightness and contrast were adjusted to improve picture quality.

Cell cycle analysis For cell cycle analysis, A549 cells were incubated with and without Nocodazole for 24 hours and harvested. After fixed with 3.8 % formaldehyde for 10 min, cells were incubated with **C61** (25 μM) or Hoechst 33342 (10 μM) for half an hour and subjected to flow cytometry (BD LSR II Bioanalyzer)



1-methyl-2,6-bis(4-(methylthio)styryl)pyridinium iodide

1,2,6-trimethylpyridinium iodide (25mg, 0.1mmol, prepared as described previously [1]) and 4-(methylthio)benzaldehyde (133mg, 1mmol) were dissolved in 5ml EtOH. 3μl of pyrrolidine was added and the reaction was heating under microwave reactor for three minutes. The solvent was removed *in vacuo*. Then, the crude was dissolved in ethyl acetate / hexane (1:1, 10ml) and filtered. After washed twice by cold ethyl acetate / hexane (1:1, 5ml × 2), C61 (34mg, 65%) was obtained as red powder. ^{1}H-NMR (DMSO-d_6): 8.38 (*t*, 1H, J=8.2Hz), 8.24 (*d*, 2H, J=8.0Hz), 7.78 (*d*, 2H, J=8.4Hz), 7.70 (*d*, 4H, J=15.9Hz), 7.63 (*d*, 2H, J=15.9Hz), 7.35 (*d*, 4H, J=8.5Hz), 4.25 (*s*, 3H), 2.52 (*s*, 6H). ESI-MS(m/z) calcd (found): 390.1 (390.0) for $[M]^+$.

References

[1]. G. R. Rosania, J. W. Lee, L. Ding, H. S. Yoon and Y. T. Chang, *J. Am. Chem. Soc.*, 2003, **125**, 1130-1131.