Supplementary Information of

A novel fluorescent probe for discriminating microbial DNAs in the ecosystems and model organisms

Zhaomin Wang [a, c], Zhe Chen [a, c] *, Hao Sun [a, c], Min Liu [b], Yong Liu [a, c] *

[a] *Institute for Ecological Research and Pollution Control of Plateau Lakes; Yunnan Key Laboratory of Plant Reproductive Adaptation and Evolutionary Ecology; School of Ecology and Environmental Sciences, Yunnan University, Kunming 650500, P.R. China*

[b] *Affiliated Hospital of Shandong University of Traditional Chinese Medicine, Jinan 250022, P.R. China*

[c] *Institute of International Rivers and Eco-Security, Yunnan University, Kunming 650500, P.R. China.*

***Corresponding author**

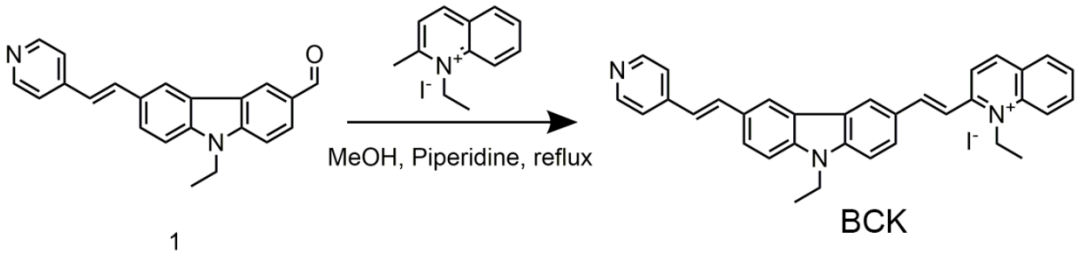
Email address: zhechen2019@ynu.edu.cn (Z. Chen)

ifp_liuy@ujn.edu.cn (Y. Liu)

Table of Contents

Materials and measurements equipment

For all experiment, all reagents were obtained by commercial suppliers. Before experiment, these reagents do not further purification. Doubly-distilled water was used throughout all the experiments. In addition, the purity of all solvents used in this work is analytical grade. Fluorescence spectra were recorded on a HITACHI F-4600 fluorescence spectrophotometer with the excitation slit widths at 5.0 nm and emission slit widths at 5.0 nm. Fig 8a was obtained by Olympus laser confocal microscope, while other fluorescence images were obtained by Zeiss laser confocal microscope.



Scheme S1 The synthetic route of probe **BCK**.

Synthesis of BCK

Synthesis routine of 1: The compound 1 was synthesized by refer literature.[1]

Synthesis routine of **BCK**: Compounds 1 (0.163 g, 0.5 mmol) and Quinaldine Ethiodide (0.179 g, 0.6 mmol) were dissolved in MeOH (100 mL). 0.2 mL piperidine was added, and the mixture was allowed to react for 12 h at 75℃ under the protection of nitrogen. The reaction solution was concentrated by vacuum distillation, and 50 mL ethyl acetate was added. After filtration, the obtained solid was washed three times with ethyl acetate. Finally solid was dried to afford the product **BCK** as a black solid with a yield of 65%. ^{1}H NMR (400 MHz, DMSO) δ 9.02 (d, *J* = 9.0 Hz, 1H), 8.92 (s, 1H), 8.69 (d, *J* = 9.1 Hz, 1H), 8.61-8.50 (m, 5H), 8.35 (d, *J* = 7.8 Hz, 1H), 8.18 (t, *J* = 8.4 Hz, 2H), 7.95 (d, *J* = 7.6 Hz, 1H), 7.92 (d, *J* = 5.8 Hz, 1H), 7.87 (s, 1H), 7.84 (s, 1H), 7.79 (d, *J* = 7.4 Hz, 1H), 7.76 (s, 1H), 7.61 (d, *J* = 5.2 Hz,

2H), 7.33 (d, *J* = 16.4 Hz, 1H), 5.23 (dd, *J* = 13.8, 6.9 Hz, 2H), 4.55 (dd, *J* = 13.4, 6.5 Hz, 2H), 1.65 (t, *J* = 7.0 Hz, 3H), 1.39 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (101 MHz, DMSO) δ 155.92, 150.47, 150.15, 145.17, 143.89, 142.64, 141.00, 138.53, 135.33, 134.22, 130.76, 129.11, 128.95, 128.79, 128.21, 126.92, 126.47, 124.21, 123.39, 123.15, 121.31, 121.10, 120.27, 119.25, 115.10, 110.82, 110.68, 46.81, 38.05, 14.66, 14.37. HRMS (m/z): $[M-I]^+$ calcd for $C_{34}H_{30}N_3^+$: 480.2434; found, 480.2443.

Species and genomic DNA (gDNA) information of Escherichia coli and Yeast

Escherichia coli str. K-12: Morphology: Gram: Negative, Shape: Bacilli, Motility: Yes, A well-studied enteric bacterium.

Lineage: Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacterales; Enterobacteriaceae; Escheri chia; Escherichia coli

Sequence information: NC_000913.3, size: 4.64Mb, GC%: 50.8

Escherichia coli: This organism is typically present in the lower intestine of humans, where it is the dominant facultative anaerobe present, but it is only one minor constituent of the complete intestinal microflora. E.coli is easily grown in a laboratory setting and is readily amenable to genetic manipulation making it one of the most studied prokaryotic model organisms. E.coli K-12 [2] is the most widely studied strain of E. coli and serves as a reference for this species.

Saccharomyces cerevisiae (baker's yeast)

Lineage: Eukaryota; Fungi; Dikarya; Ascomycota; Saccharomycotina; Saccharomycetes; Saccharomycet ales; Saccharomycetaceae; Saccharomyces; Saccharomyces cerevisiae

Size: 11.83 Mb; GC%: 38.2

The budding yeast *Saccharomyces cerevisiae* is one of the major model organisms for understanding cellular and molecular processes in eukaryotes. This single-celled organism is also important in industry, where it is used to make bread, beer, wine, enzymes, and pharmaceuticals. The *Saccharomyces cerevisiae* genome is approximately 12 Mb, organized in 16 chromosomes.

The selective and competitive experiments

The configuration method of probe mother liquor and DNA mother liquor is the same as that of titration experiment. Other ions and small molecules in testing were prepared by dissolving the

following compounds in water: $NaAC·3H_2O$, $CuCl_2·2H_2O$, NaF, $Na_3PO_4·12H_2O$, KCl, NaCl, $BaCl_2·2H_2O$, Na_2SO_4, $CaCl_2$, cysteine (Cys). $FeSO_4·7H_2O$, $NiCl_2·6H_2O$, $MgCl_2$, NaBr, $ZnSO_4·7H_2O$, KNO_3, $NaNO_2$. In the selective test, different test solutions were diluted to 1mM (the concentration of Cys is 0.1 mM) with Tris-HCl buffer solution. 1.99 mL diluted solution was taken into a centrifuge tube, 10 μL probe **BCK** mother liquor was added, and the centrifuge tube was shake well. A group of test samples containing different ions or small molecules were obtained. The fluorescence spectra of each sample were tested respectively. In the competitive experiments, different test solutions were diluted to 2 mM (the concentration of Cys is 0.2 mM) with Tris-HCl buffer solution. 0.995 mL diluent was mixed with the 0.995 mL DNA solution (2 mM), and 10 μL **BCK** (1 mM) was added. After shaking, the fluorescence spectrum of each sample was tested.

Cell culture and imaging

The HepG2 cells were cultured in a petri dish in a constant temperature incubator at 37 ℃ and 5% carbon dioxide. The components of the medium were DMEM and 10% DMF. The HepG2 cells were transferred to an imaging dish for culture before imaging. When the cells cover about 80% of the area of the imaging dish, the cells were treated and imaged. First, the cells were treated with 4% formalin solution for 30 minutes to fix the cells on the imaging dish. Then, formalin solution was removed and 0.4% Triton X-100 was added to treat cells for 2 min. Finally, after the cells were incubated with the probe **BCK** (10 μM) for 30 minutes, the cells were washed twice with PBS for imaging.

Ecoli. and Yeast imaging were further conducted by Olympue optical microscope. First, Ecoli. and yeast cells were incubated with the probe **BCK** (5 μM) for 20 minutes Second, both cells were coated on the glass slides. Then the Ecoli. and yeast cells imaging experiments were prepared by the red (λ_{ex} = 560-600 nm, λ_{em} = 630–706 nm) than the blue (λ_{ex} = 350-400 nm, λ_{em} = 460–510 nm) and green emission channels (λ_{ex} = 480-520 nm, λ_{em} = 527–557 nm) under the same condition. Control experiments

for the cell experiments were all performed under the same conditions.

RNase digestion imaging experiment

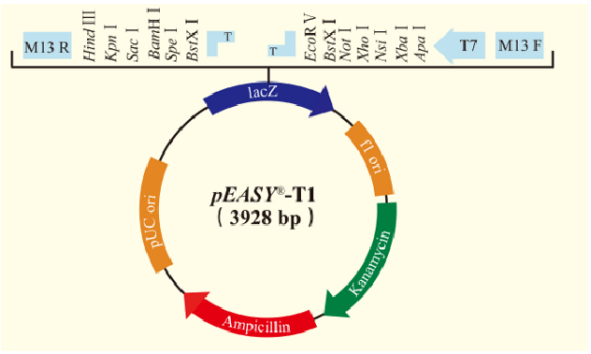
The fixed cells were pierced with 1 % Triton X-100 for 5 min and washed with PBS. Then, the cells were divided into two groups. The cells in the experimental group were treated with PBS containing RNase (30 mg/ mL) or DNase (10 u/ mL) and $MgCl_2$ for 3 h. After washing again, the two groups of fixed HepG2 cells were incubated with the probe **BCK** and imaged.

Lysosomes co-localization fluorescence images

The fixed HepG2 cells were inoculated into a 35 mm Petri dish and incubated for 24 h. The **BCK** (10 μM) was added to the cells and incubated 15 min at 37℃. The commercially available mitochondrial probe Lyso Tracker Deep Red (5 μM) was incubated with the cells for 15 min, and then washed three times with PBS. Finally, imaged with ZEISS fluorescence microscope.

Nucleus co-localization fluorescence images

The fixed HepG2 cells were inoculated into a 35 mm Petri dish and incubated for 24 h. The **BCK** (10 μM) was added to the cells and incubated 20 min at 37℃. Then, DAPI (1 μM) was added and co-incubated with the cells for 10 min, and then washed three times with PBS. Finally, imaged with ZEISS fluorescence microscope.



3928bp circular double-strand DNA

1050bp linear double-strand DNA

1050bp linear single-strand DNA

Fig. S1 Schematic diagram of DNA three structures.

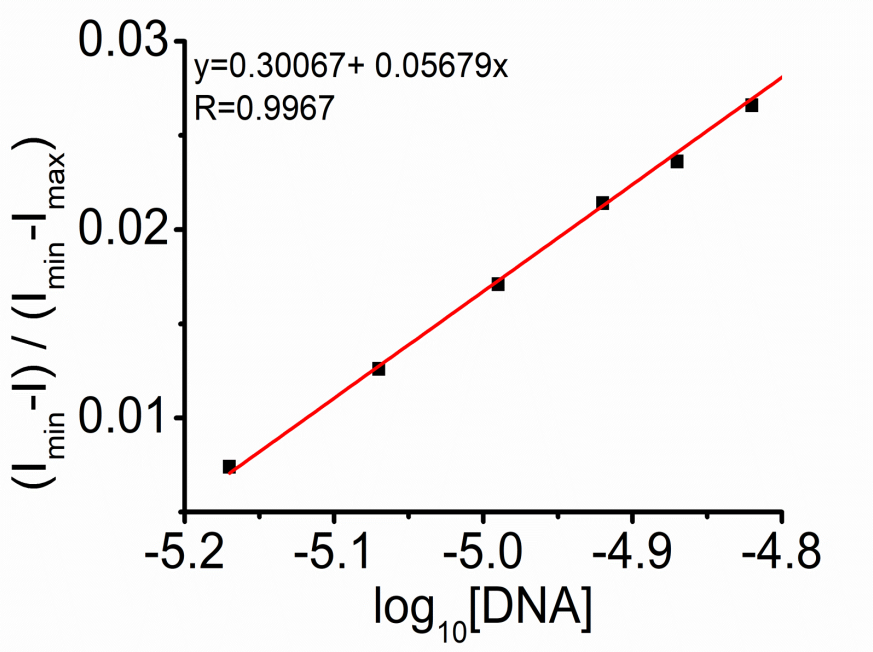


Fig. S2 The limit of detection for DNA.

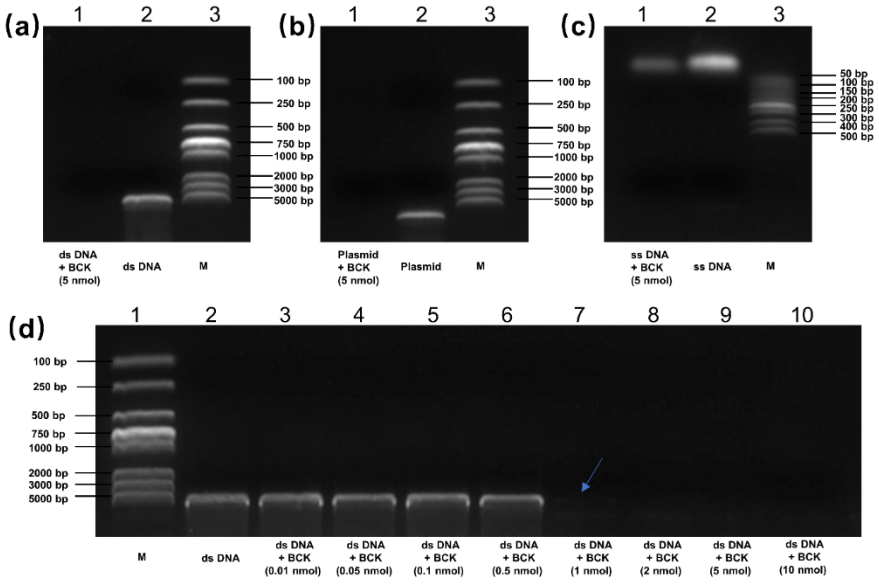


Fig. S3 (a) Agarose gel electrophoresis diagram of probe binding with double-stranded DNA. Lane 1, ds DNA and 5 nmol **BCK**; Lane 2, ds DNA; Lane 3, DNA molecular standard. (b) Agarose gel electrophoresis diagram of probe binding with plasmid. Lane 1, plasmid and 5 nmol **BCK**; Lane 2, plasmid; Lane 3, DNA molecular standard. (c) Agarose gel electrophoresis diagram of probe binding with single-stranded DNA. Lane 1, ss DNA and 5 nmol **BCK**; Lane 2, ss DNA; Lane 3, DNA molecular standard. (d) Agarose gel electrophoresis diagram of probe binding with double-stranded DNA. Lane 1, DNA molecular standard; Lane 2, ds DNA; Lanes 3-10, ds DNA and 0.01 nmol, 0.05 nmol, 0.1 nmol, 0.5 nmol, 1 nmol, 2 nmol, 5 nmol, 10 nmol **BCK**.

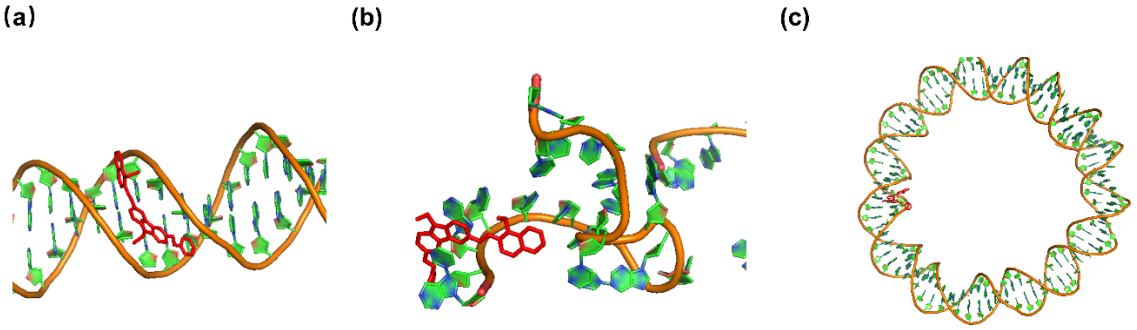


Fig. S4 The binding mode of BCK to the crystallized structure of double-stranded DNA (RCSB Protein Data Bank ID: 8UCW) [3], single-stranded nucleic acid (RCSB Protein Data Bank ID: 4OE1) [4], and circular DNA (RCSB Protein Data Bank ID: 6UPH) [5]. The affinity of BCK to double-stranded DNA, single-stranded nucleic acids, and circular DNA is -10.96 kcal/mol, -7.4 kcal/mol, and -10.69 kcal/mol, respectively.

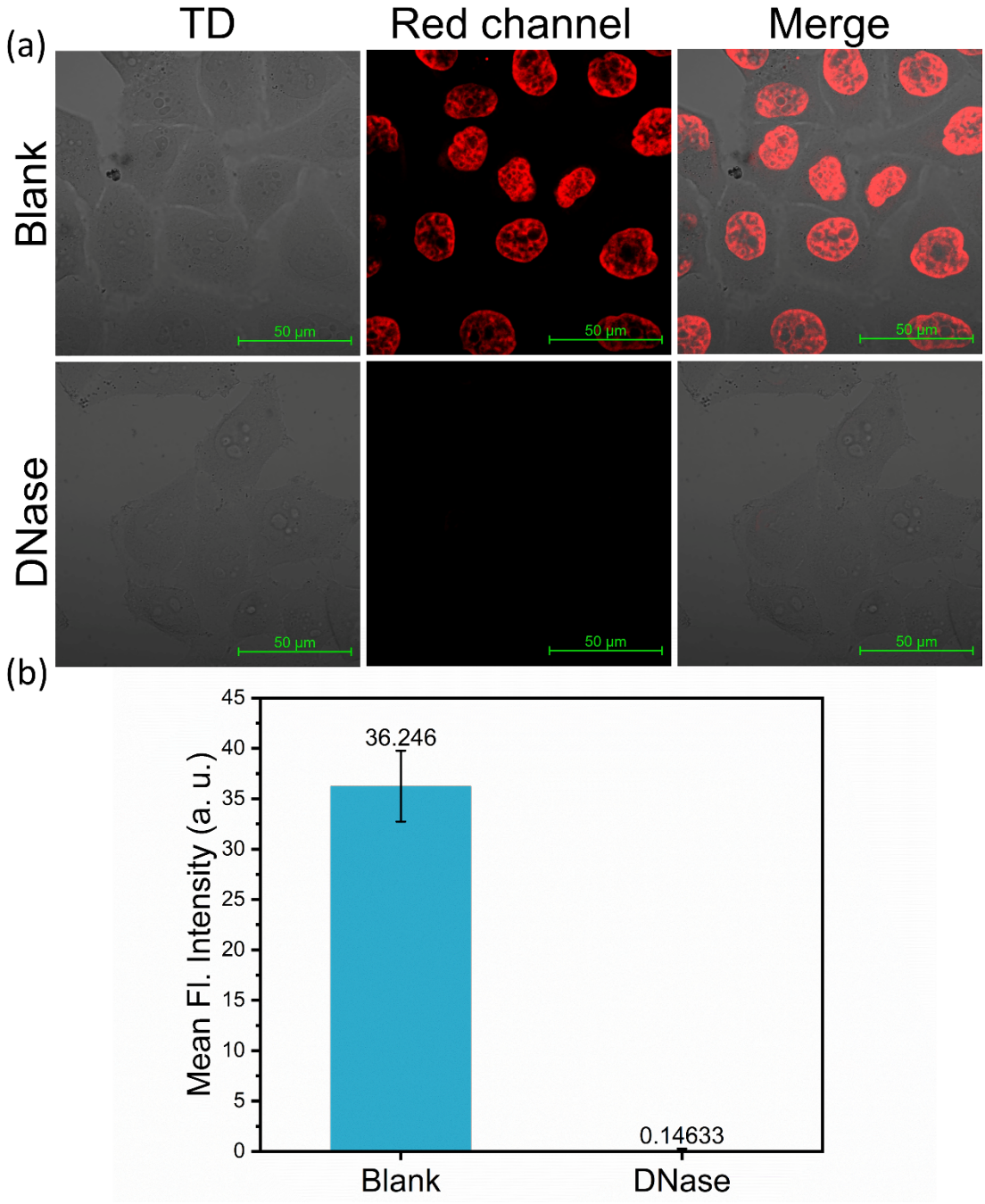


Fig. S5 DNase enzyme digestion experiment conducted in HepG2 cells. (a) Imaging of cells before and after DNase enzyme digestion. (b) Average fluorescence intensity within cells before and after DNase enzyme digestion.

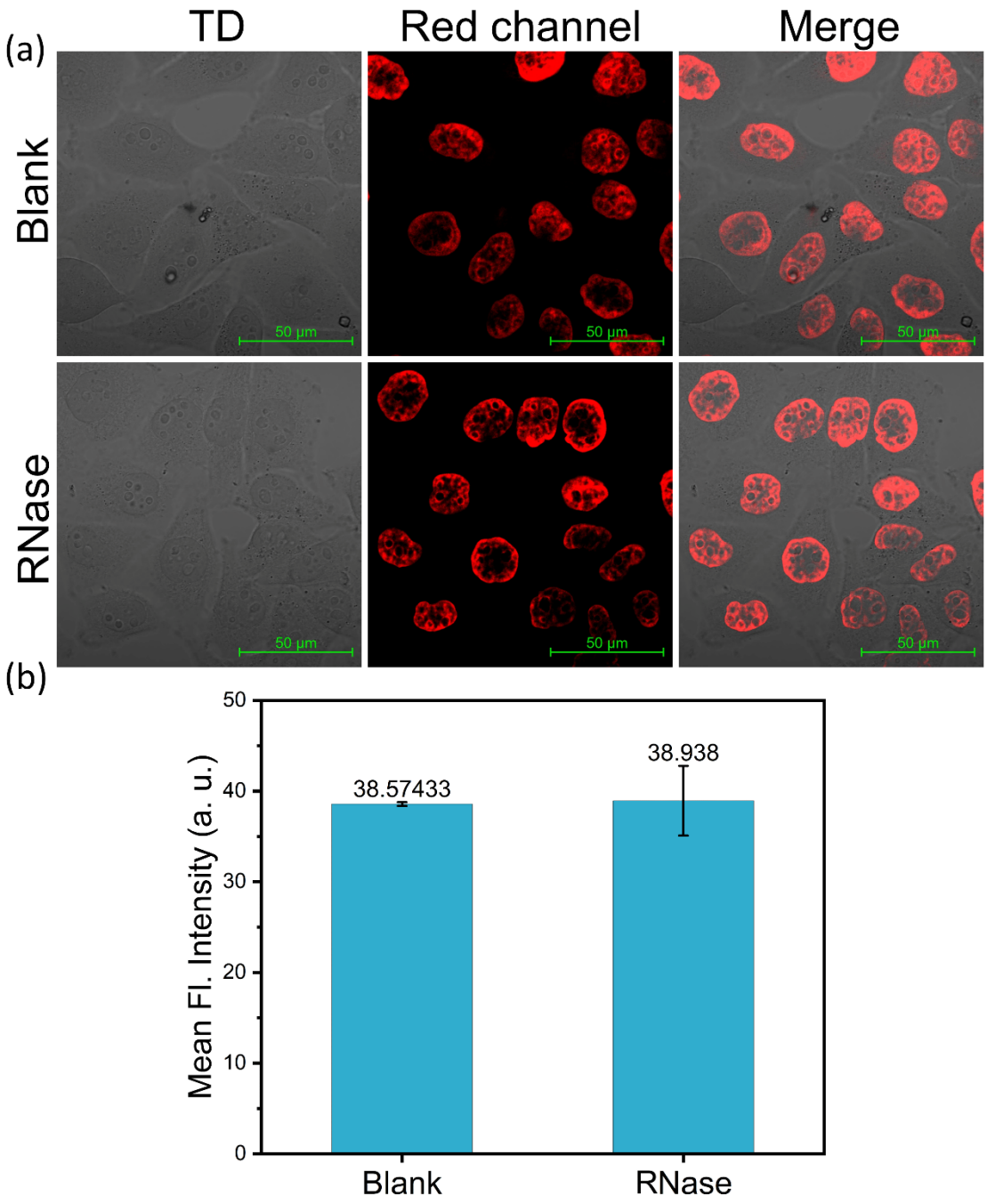


Fig. S6 RNase enzyme digestion experiment conducted in HepG2 cells. (a) Imaging of cells before and after RNase enzyme digestion. (b) Average fluorescence intensity within cells before and after RNase enzyme digestion.

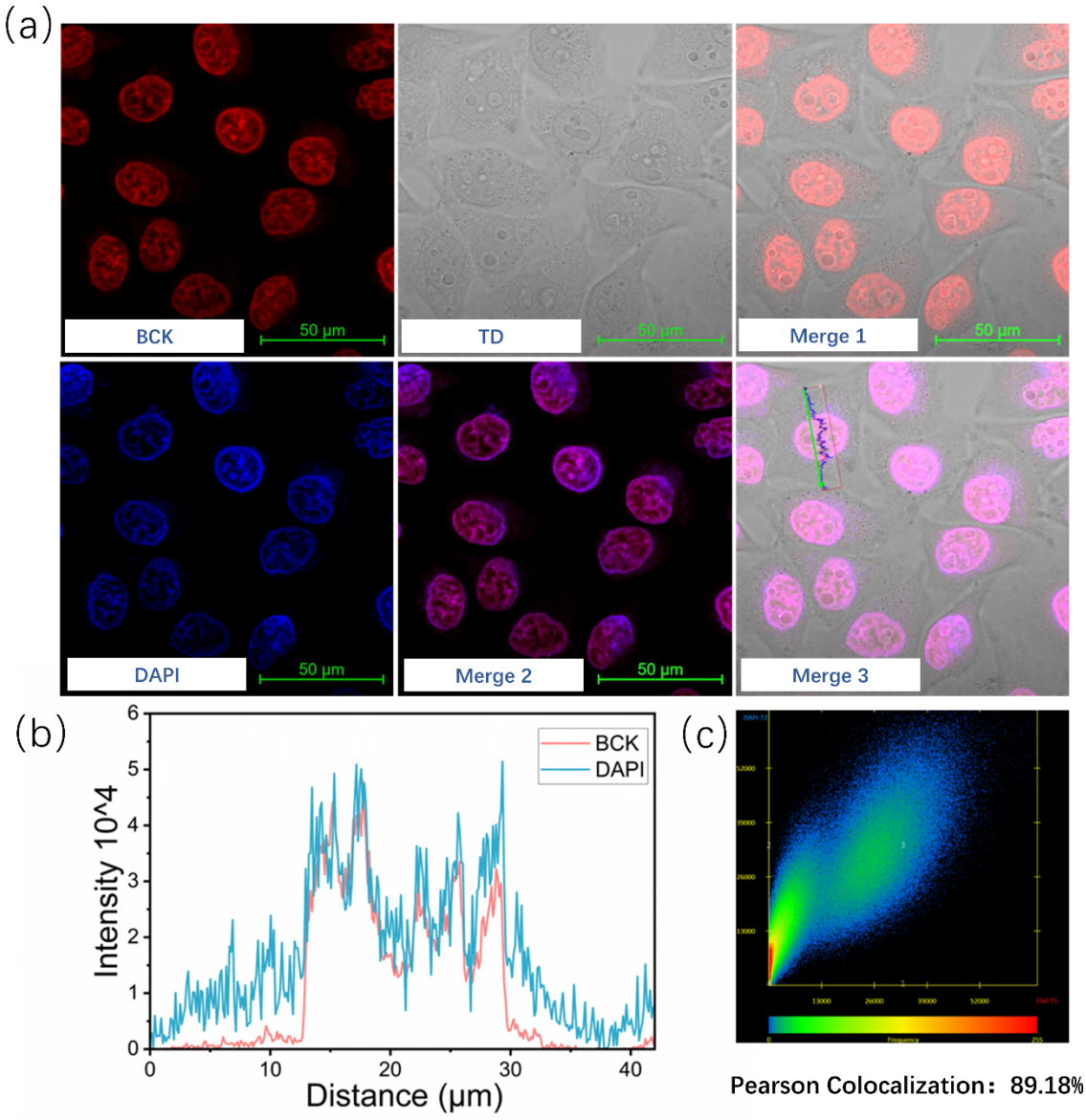


Fig. S7 The DNA co-localization experiment is used to eliminate potential interference within the cell. **(a) Co-staining experiment of BCK and DAPI in cells; (b The trend of fluorescence intensity of BCK and DAPI along the white arrow in Fig. S7 (a); (c) Scatter plot and Pearson's co-localization coefficient of DAPI and BCK in Fig. S7 (a).**

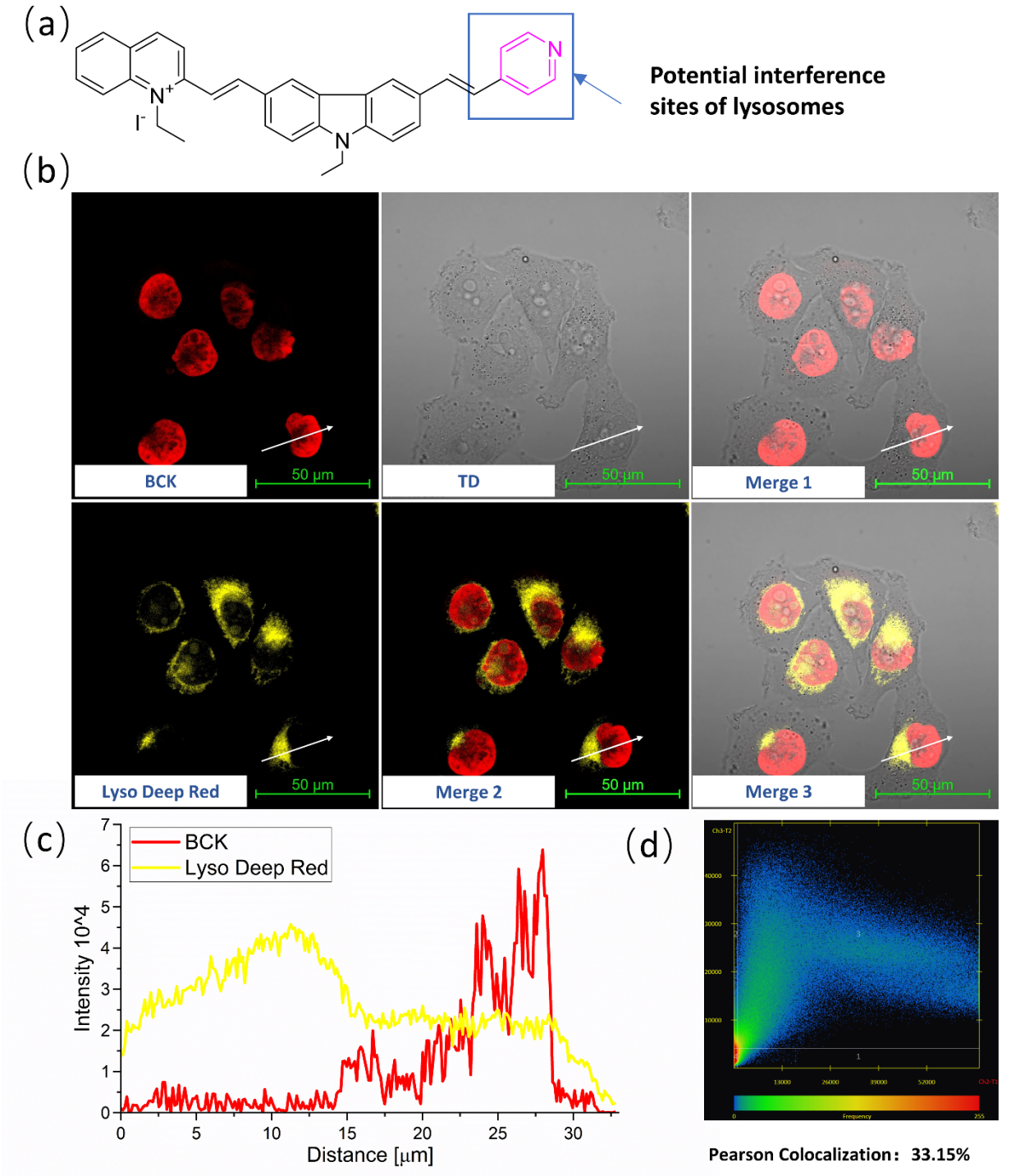


Fig. S8 The lysosome co-localization experiment is used to eliminate potential interference within the cell. (a) Potential lysosomal interference sites in the **BCK** structure; (b) Co-staining experiment of **BCK** and lysosome deep red dye in cells; (c) The trend of fluorescence intensity of **BCK** and lysosome deep red along the white arrow in Fig. S8 (b); (d) Scatter plot and Pearson's co-localization coefficient of lysosome deep red and **BCK** in Fig. S8 (b).

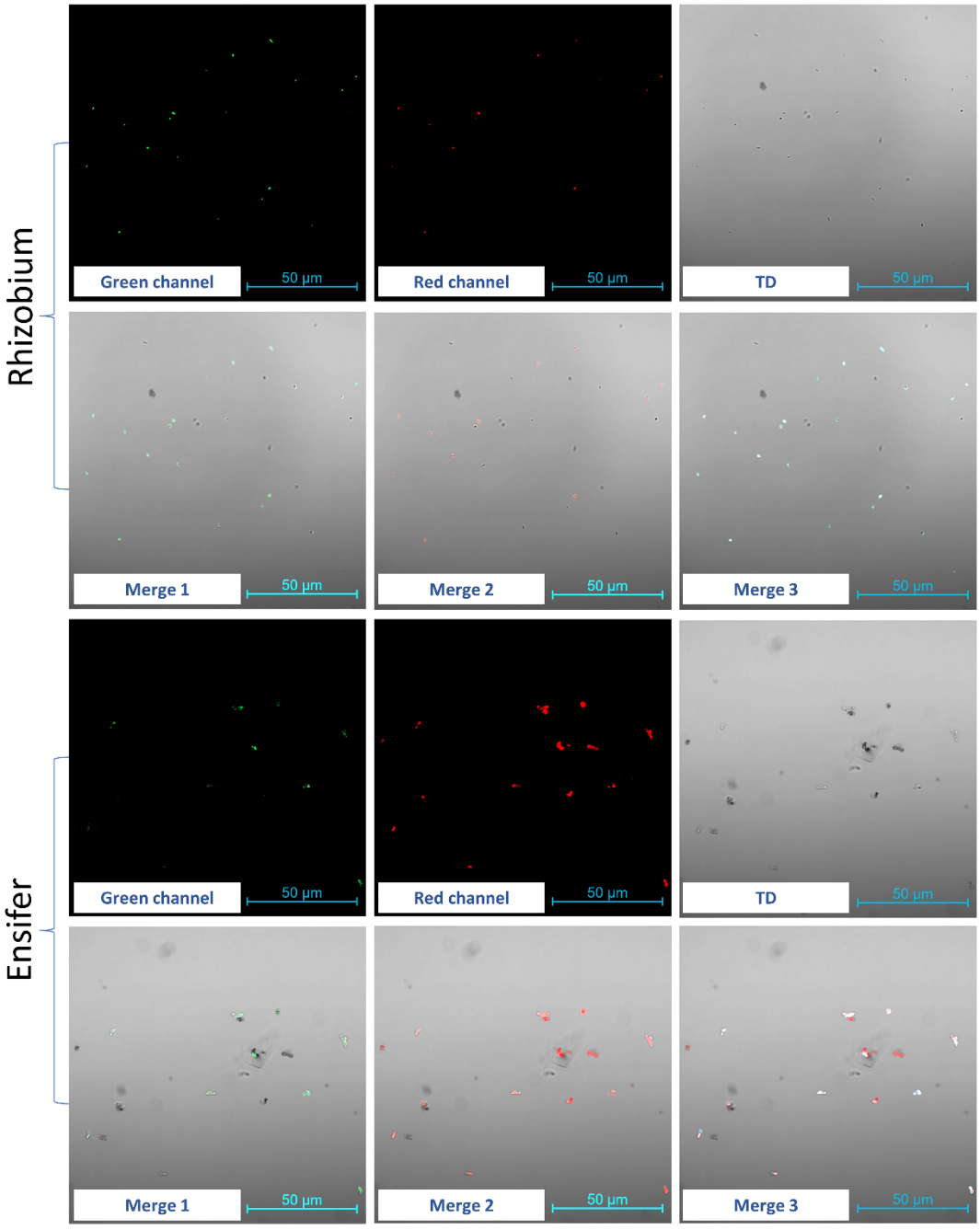


Fig. S9 Imaging of **BCK** in two different genera (Rhizobium and Ensifer) of bacteria isolated from root nodules of leguminous plants in cropland.

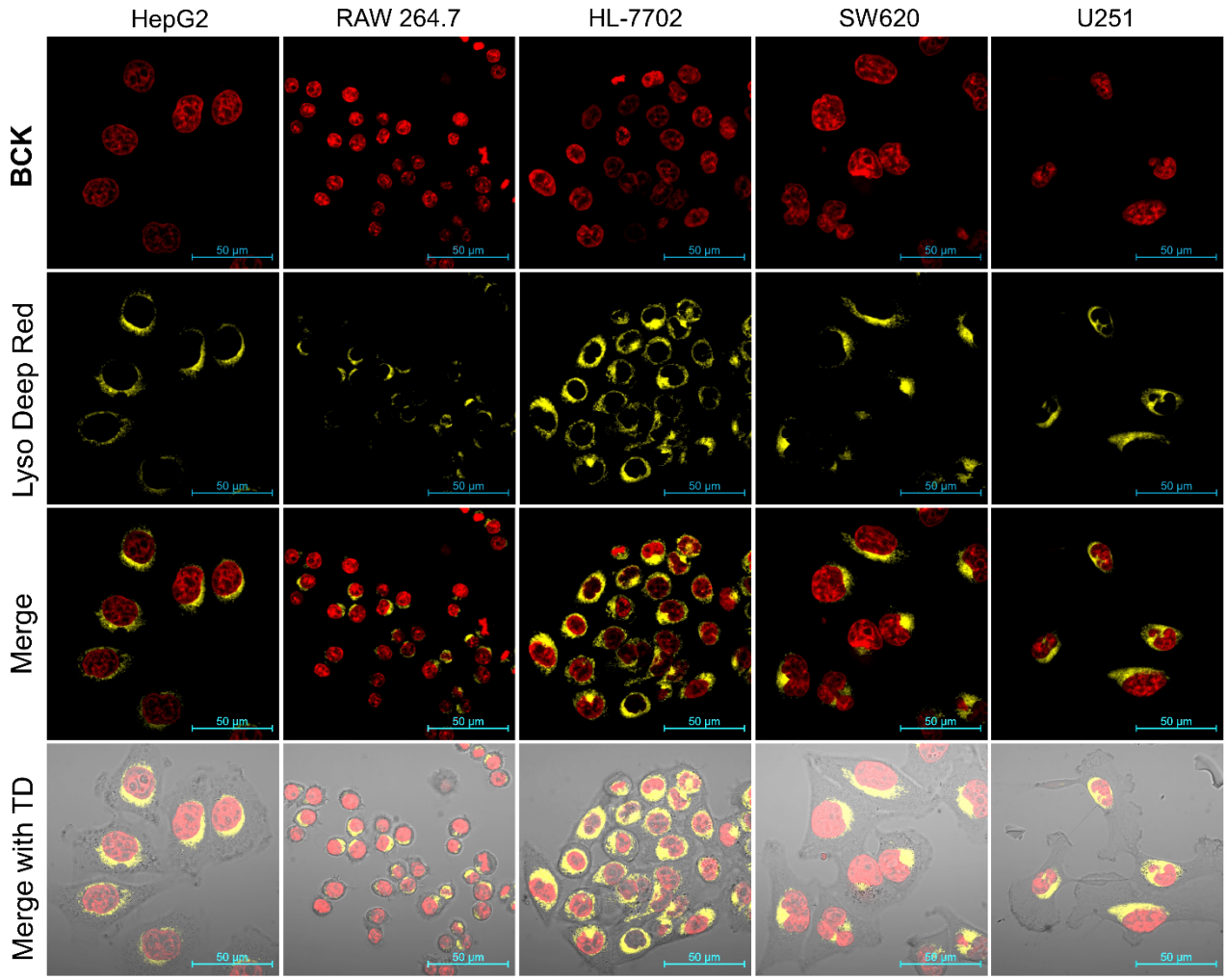


Fig. S10 Imaging of **BCK** and lysosomes deep red dyes in fixed HepG2, RAW 264.7, HL-7702, SW620 and U251 cells.

Characterization

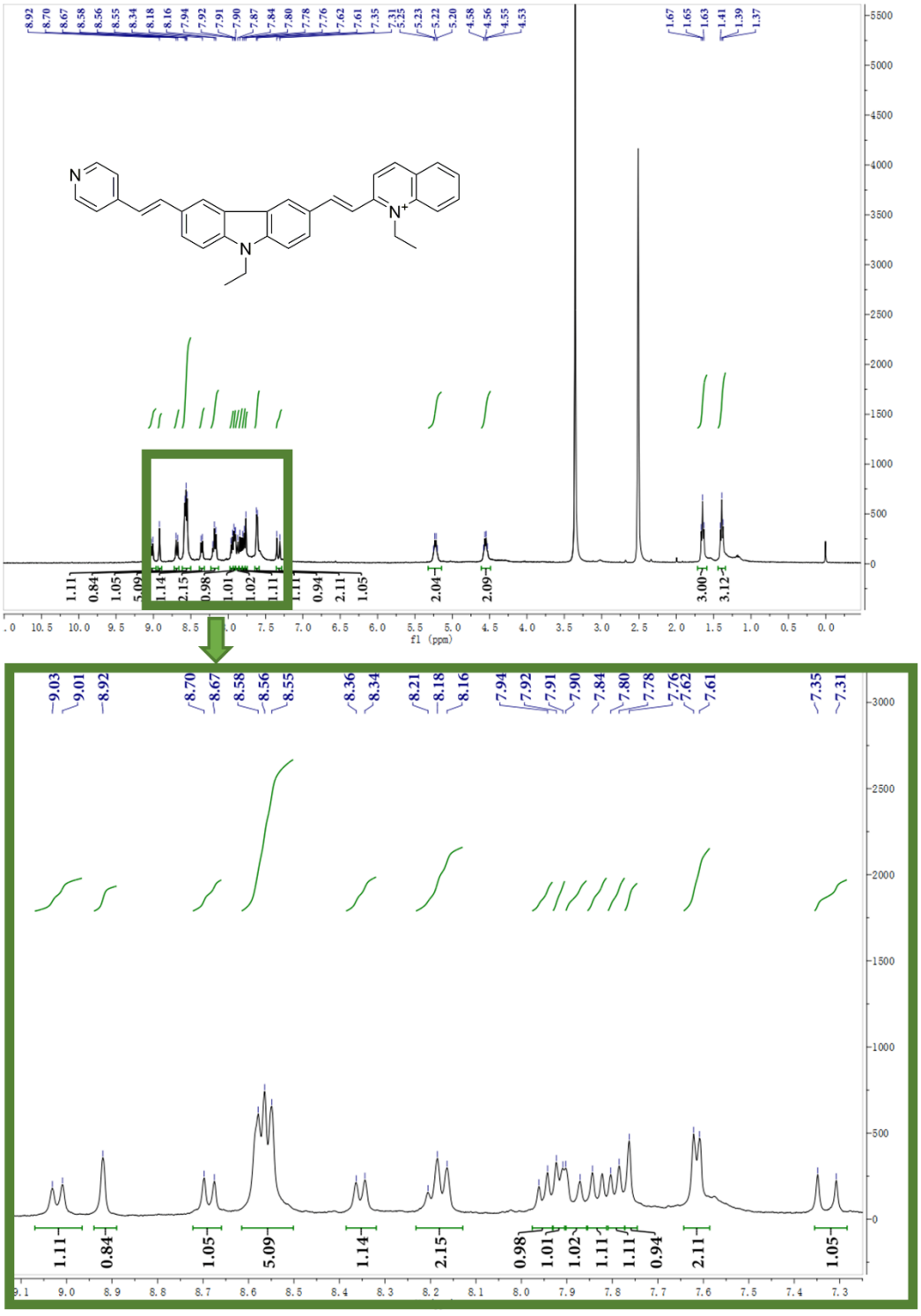


Fig. S11 The ^{1}H NMR spectrum of the compound **BCK** in DMSO-*d6*.

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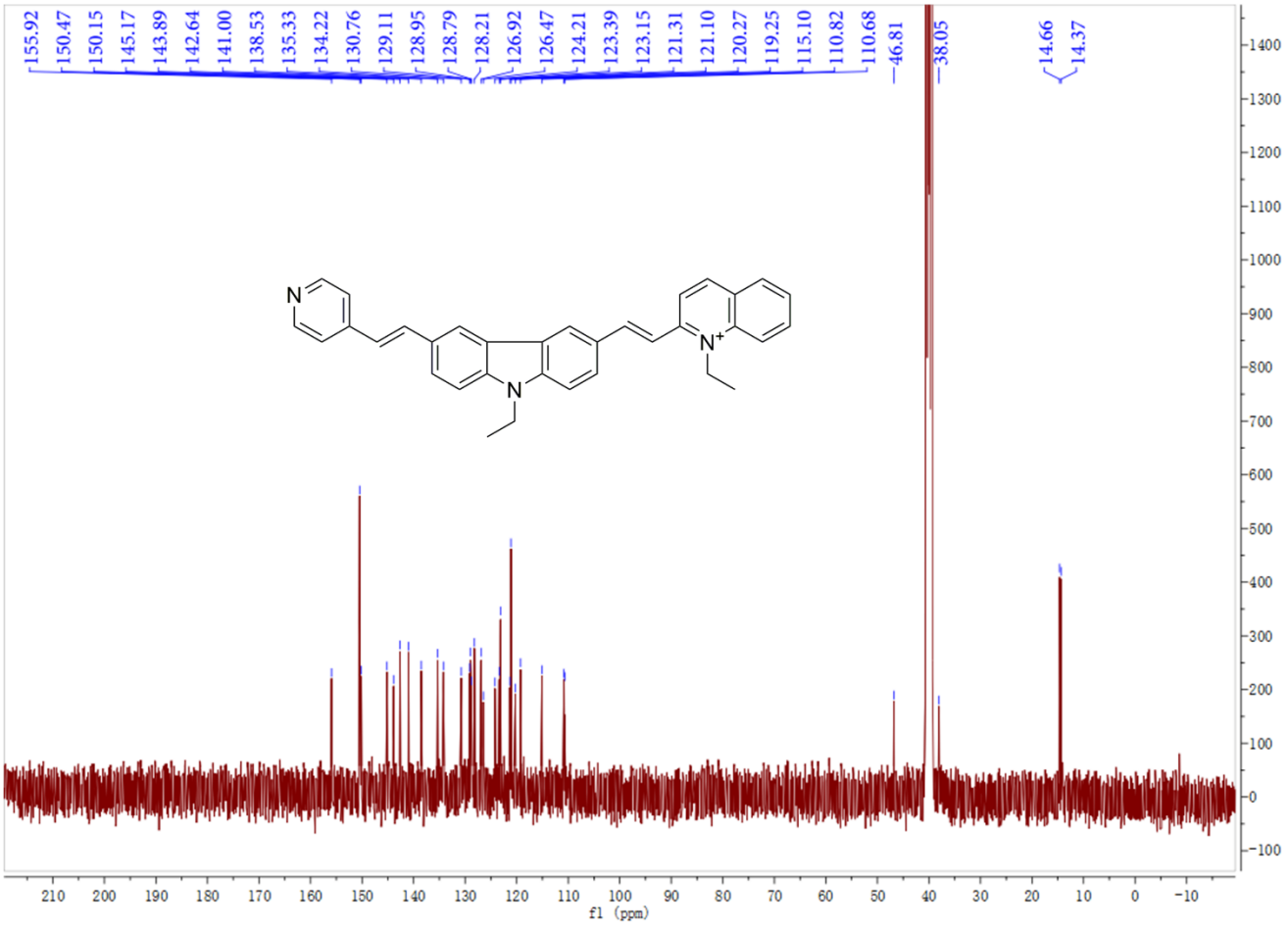

Fig. S12 The ^{13}C NMR spectrum of the compound **BCK** in DMSO-*d6*.

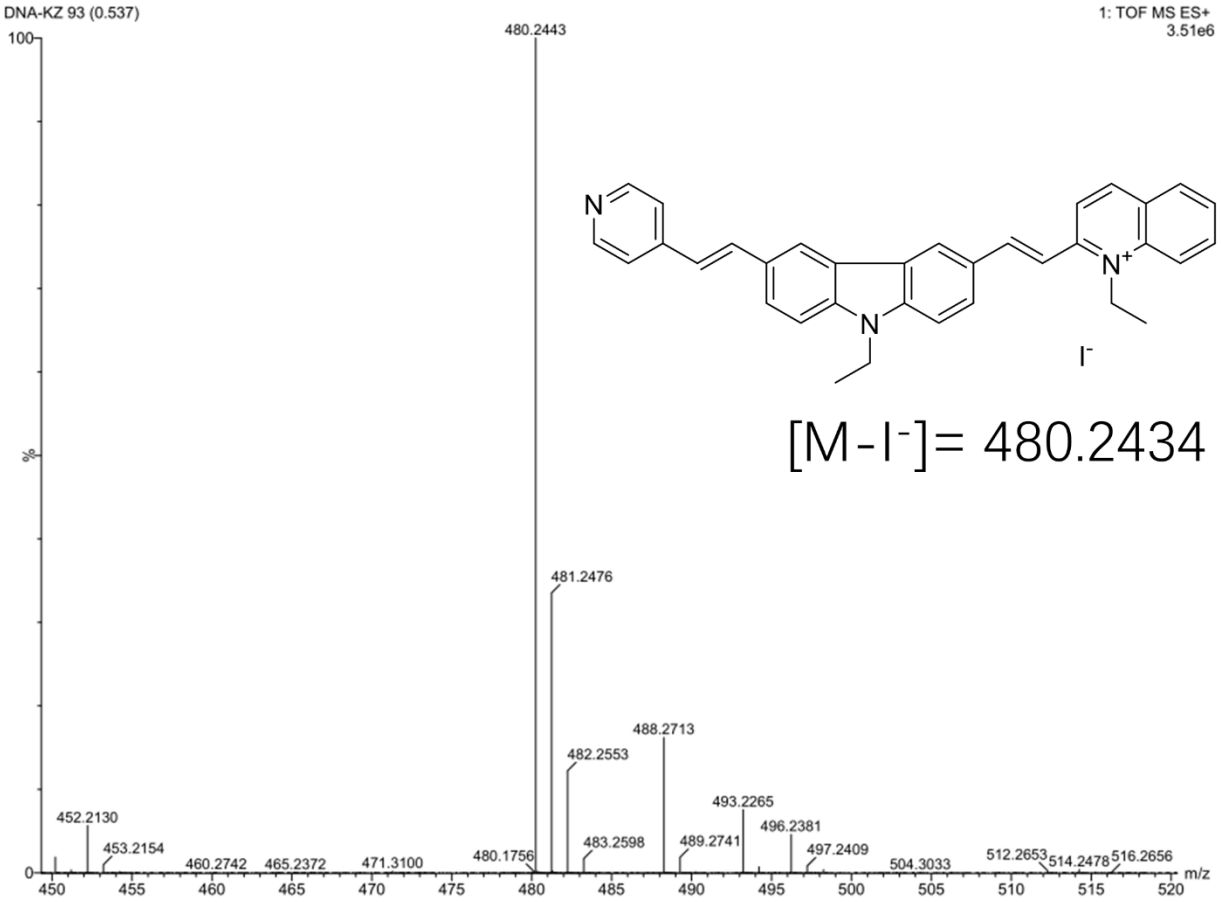

Fig. S13 The HRMS spectrum of the compound **BCK**.

References

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