

Supplementary Information

Cysteine and Viscosity double-locked response fluorescent probe for imaging in HeLa cells

Yuting Du*, Minmin Guo, Xingyu Xu, Sanhu Zhao, Jie Yang

*Department of Chemistry, Xinzhou Normal University, Xinzhou, Shanxi 034000,
China*

*Corresponding author.

E-mail: yutingdu123@163.com

Synthesis of Compound CDFN-OH

Synthesis of **CDFN-OH**. 2-(3-cyano-4,5,5-trimethylfuran-2(5H)-ylidene)malononitrile (200 mg, 1 mmol and 6-hydroxy-2-naphthaldehyde (170 mg, 1 mmol) were dissolved in 20 mL of absolute ethanol under ultrasonic bath. Then acetic acid (50 mg, 0.88 mmol) and acetic anhydride (110 mg, 1.1 mmol) were added to the solution. The reaction were stirred and refluxed at 90 °C for 9 h. After the reaction is completed, it was poured into 10 mL of water and extraction with dichloromethane. The obtained sample was purified by silica gel chromatography with $v(\text{CH}_2\text{Cl}_2)/v(\text{CH}_3\text{OH})$ (60:1) as the eluent to yield **CDFN-OH** as a crimson solid (212.0 mg, 60%).

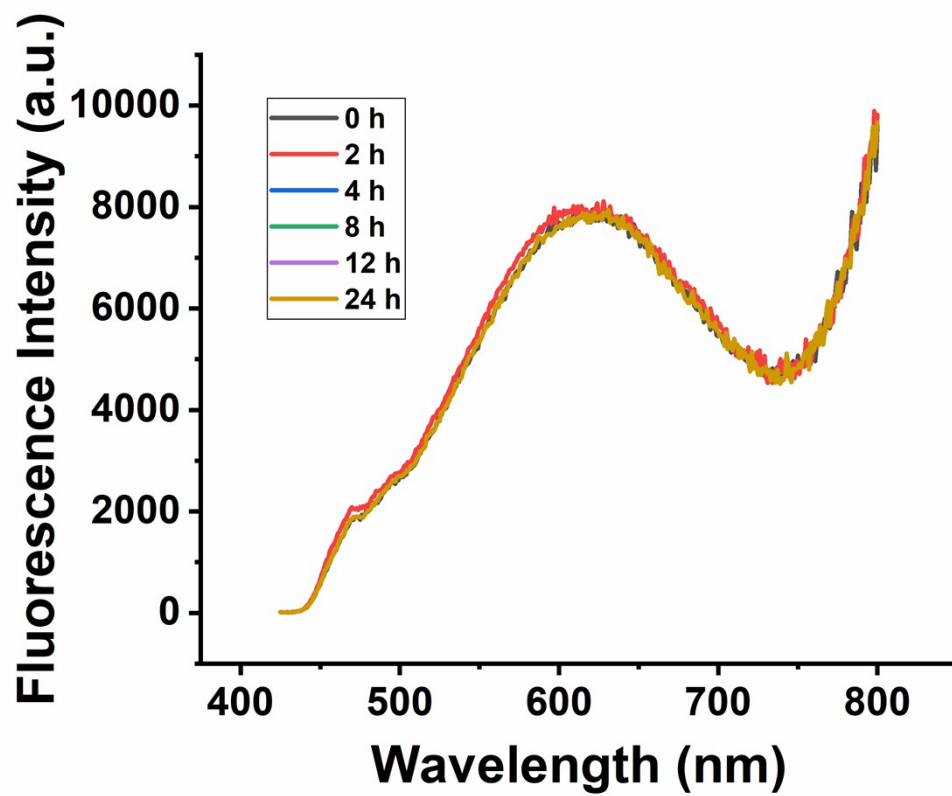


Figure S1. The stability of the probe CDFN-ACY in physiological buffer solutions.

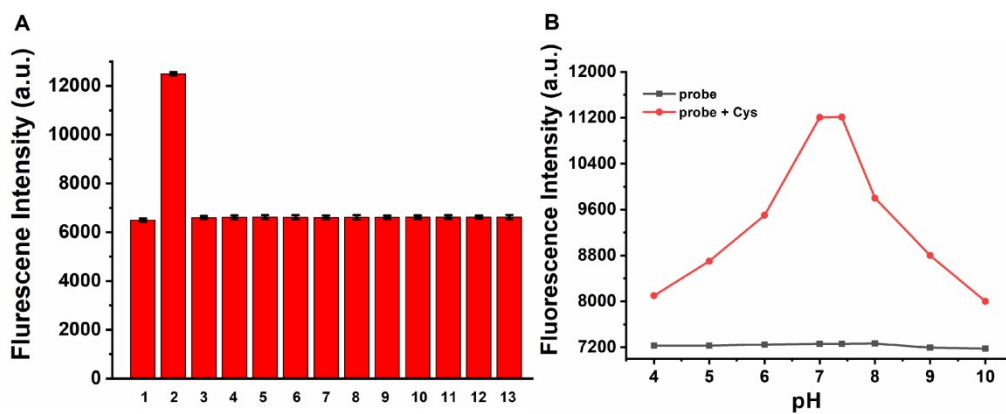


Figure S2. (A) Fluorescence emission intensity of **CDFN-ACY** (5 μM) to Cys (300 μM), and other interfering substances (1 mM), including 1: blank, 2: Cys, 3: GSH, 4: Hcy, 5: NO₃⁻, 6: NO₂⁻, 7: PO₄³⁻, 8: SO₄²⁻, 9: SO₄²⁻, 10: Zn²⁺, 11: Cu²⁺, 12: K⁺, 13: Na⁺. (B) pH-dependent fluorescence emission of **CDFN-ACY** (5 μM) in response to Cys (300 μM) in CH₃CN/PBS medium (v/v = 1:1, 10.0 mM).

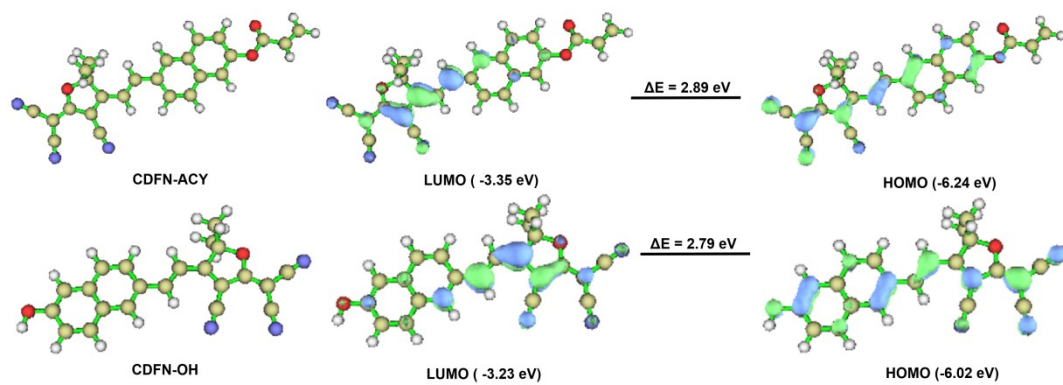


Figure S3. Structural optimization and the molecular orbitals of probe **CDFN-ACY** and **CDFN-OH**.

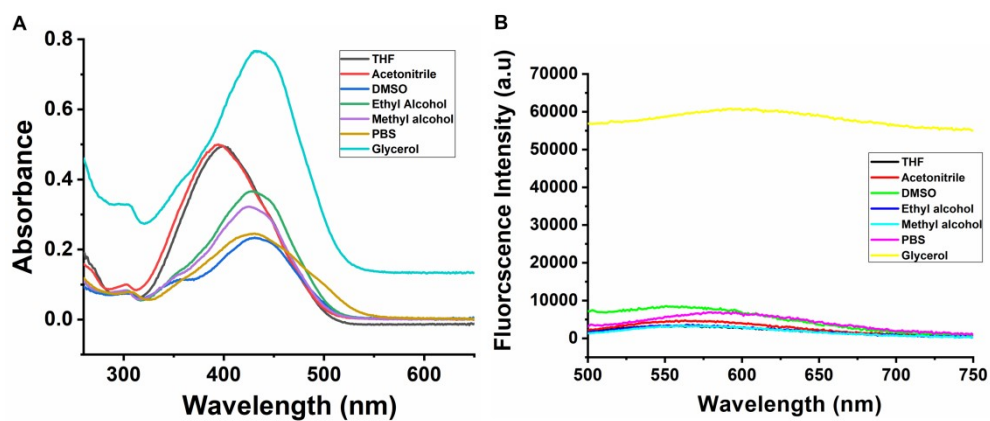


Figure S4. The UV-vis absorption and fluorescence spectra of **CDFN-ACY** upon the addition of Cys in different solvents.

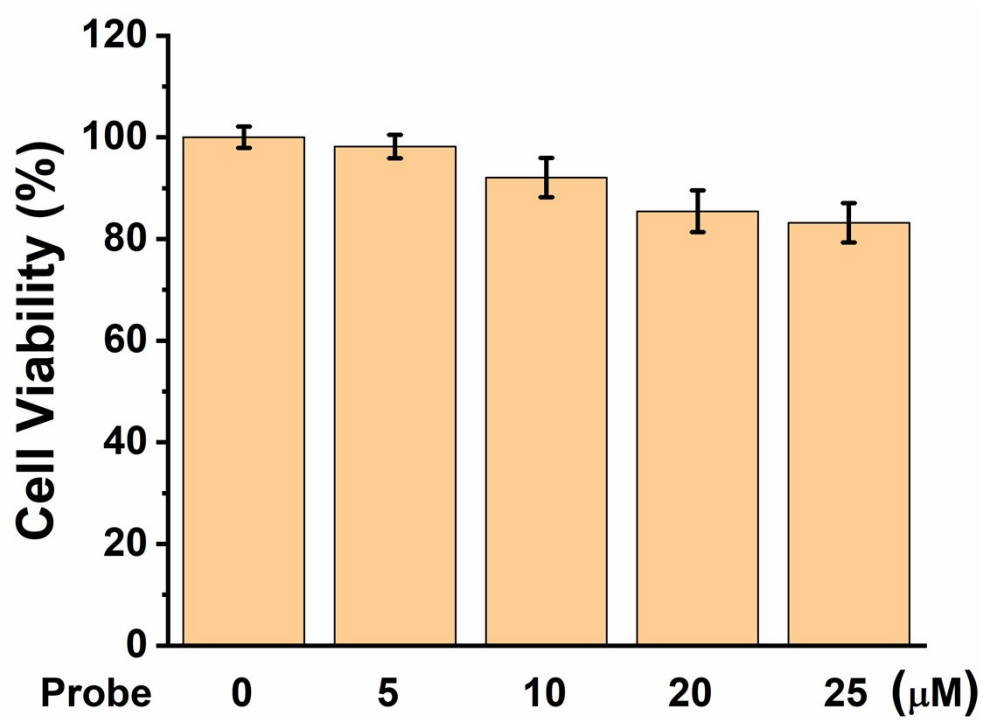


Figure S5. Cell viability of HeLa cells treated with different concentrations of CDFN-ACY for 24 h.

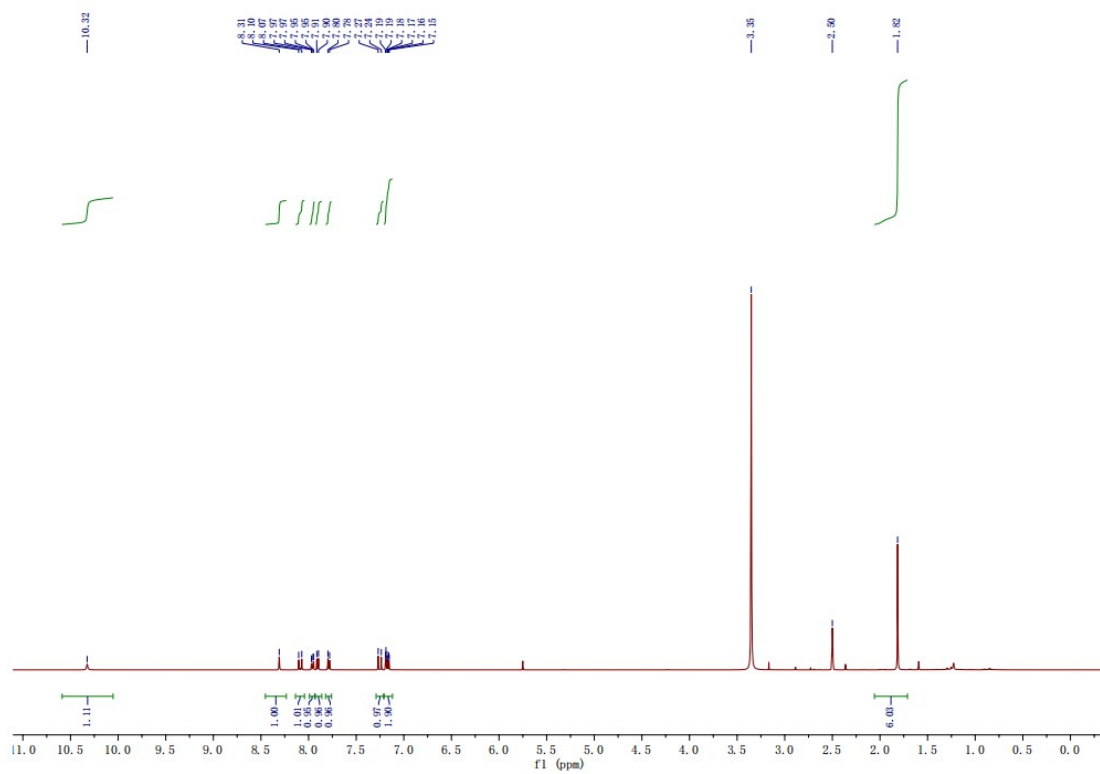


Figure S6. ^1H NMR spectra of CDFN-OH in $\text{DMSO-}d_6$.

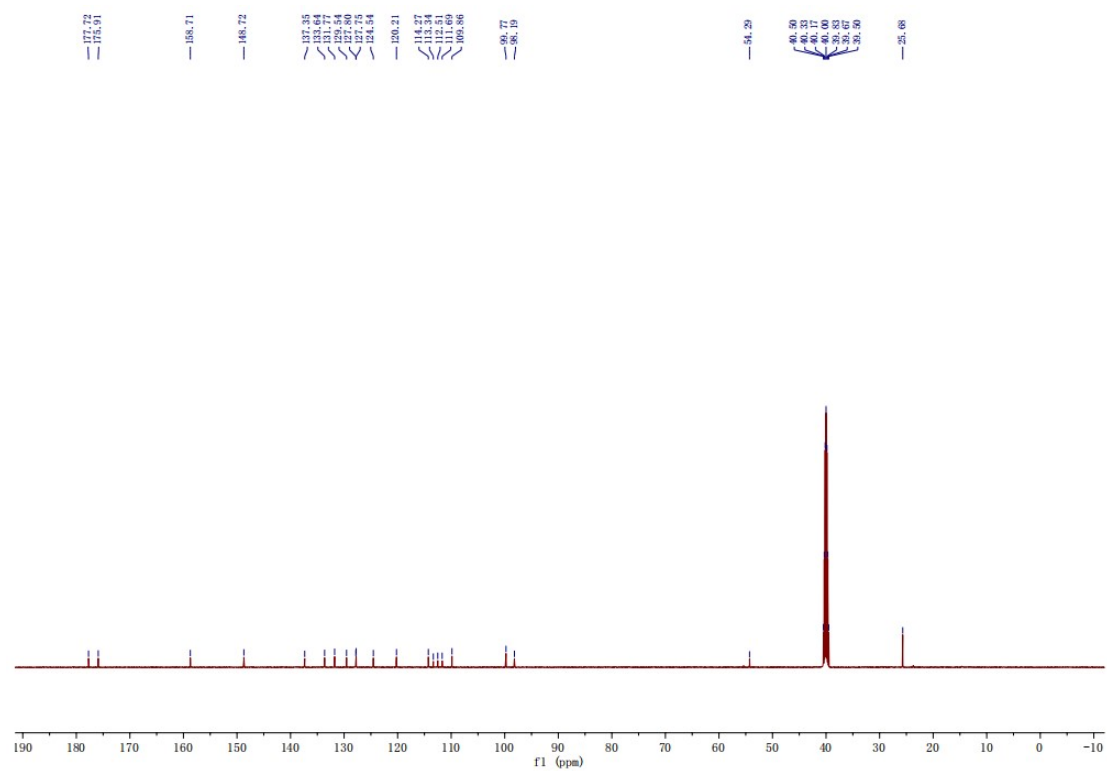


Figure S7. ^{13}C NMR spectra of CDFN-OH in $\text{DMSO-}d_6$.

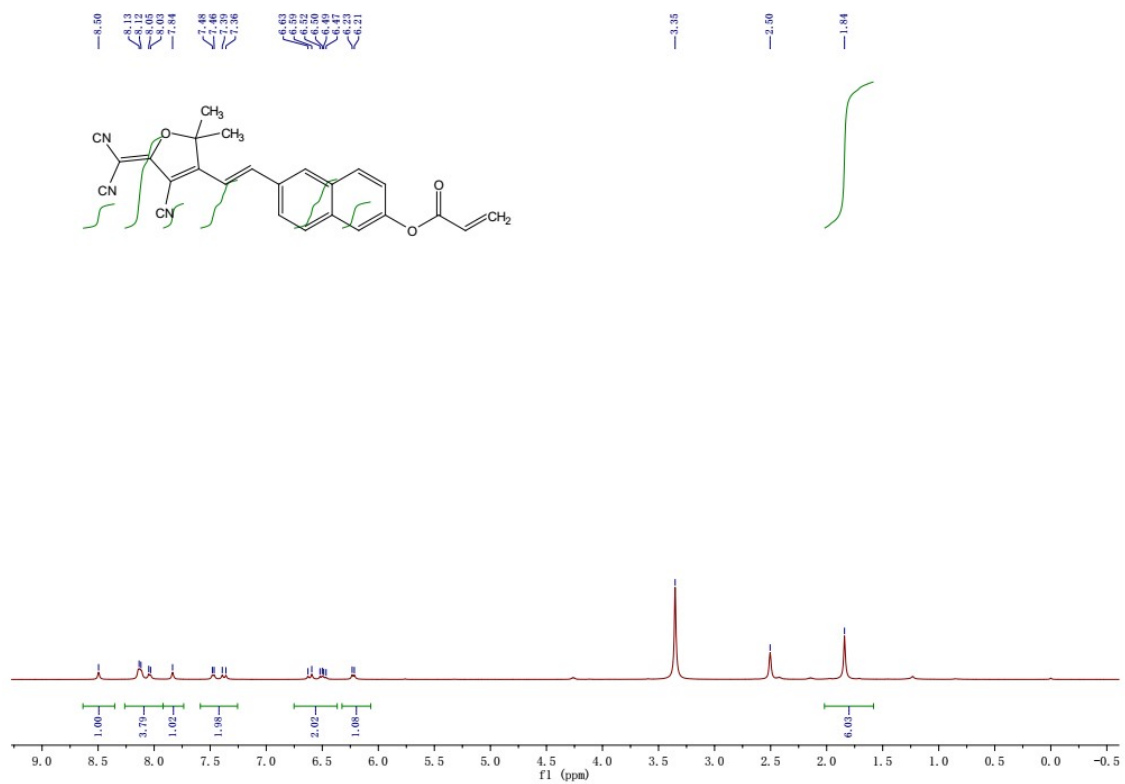


Figure S8. ¹H NMR spectra of CDFN-ACY in DMSO-*d*₆.

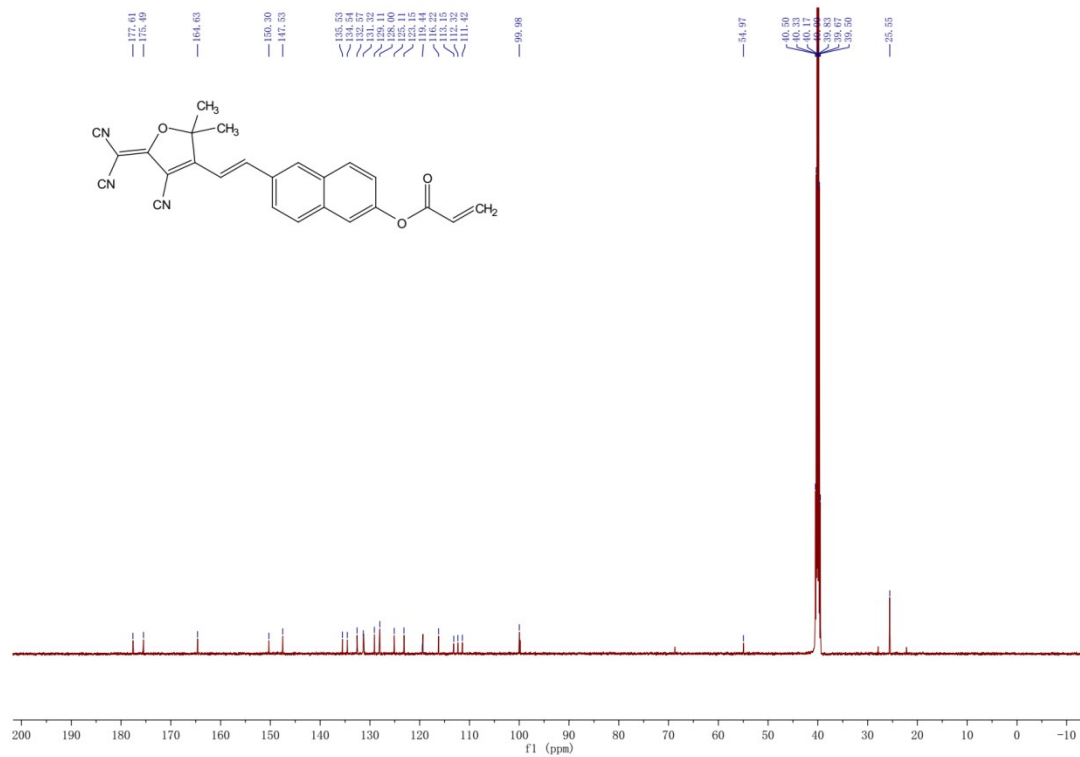


Figure S9. ¹³C NMR spectra of CDFN-ACY in DMSO-*d*₆.

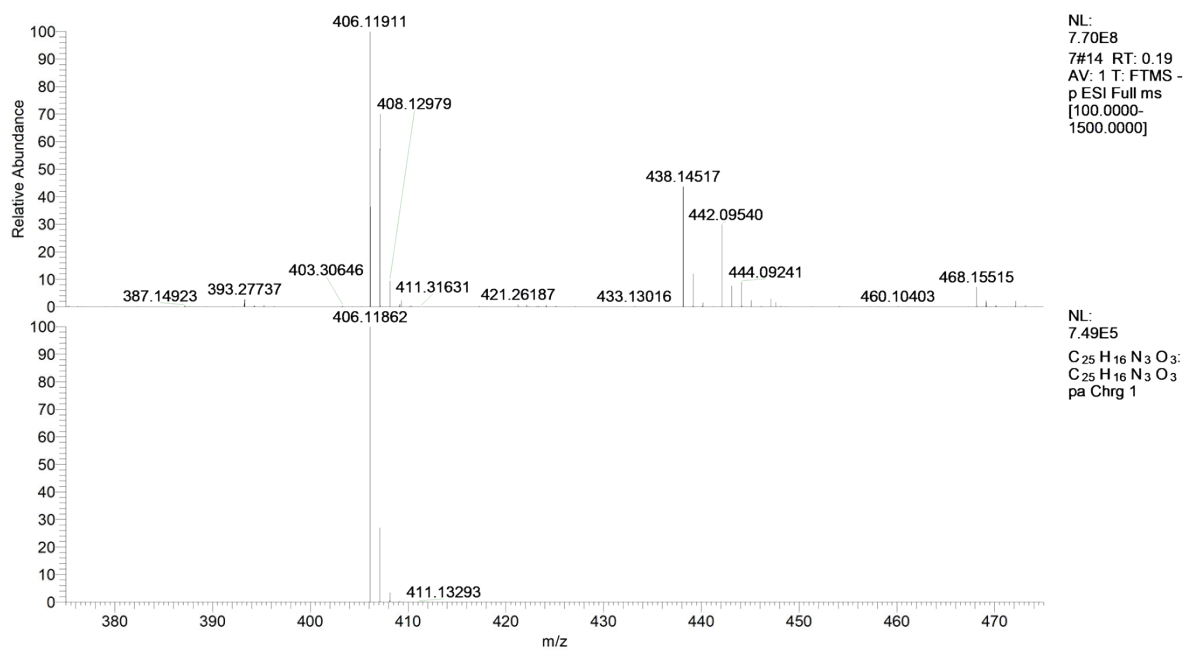


Figure S10. HRMS spectra of CDFN-ACY.