Electronic Supplementary Material

In-situ hydrophobic environment triggering reactive fluorescence probe to real-time monitor mitochondrial DNA damage

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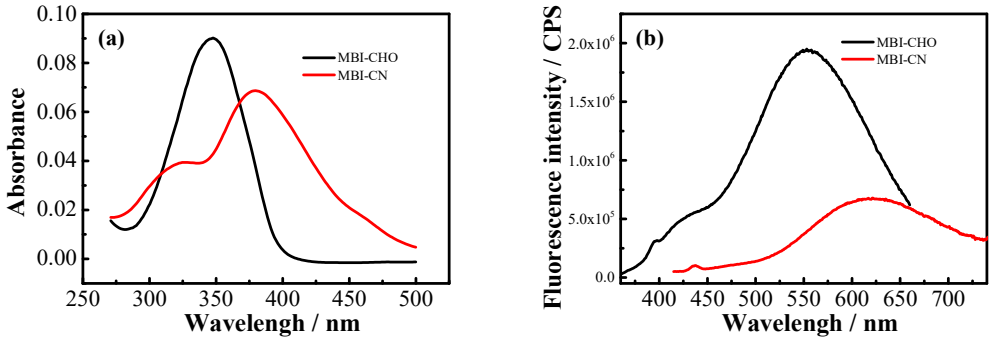


Fig. S1 Absorption and emission of MBI-CN and MBI-CHO in water.

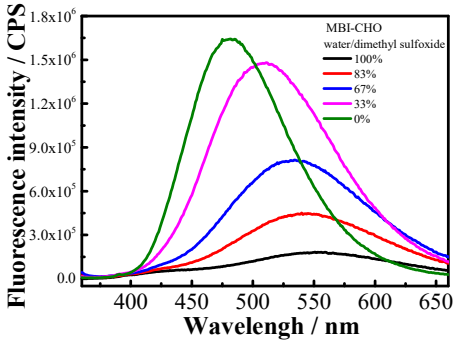


Fig. S2 The fluorescence spectra of MBI-CHO in different water and dimethyl sulfoxide mixed solutions with hydrophilicity and hydrophobicity.

Mechanism of hydrolysis of probe MBI-CN

The hydrolysis of MBI-CN to MBI-CHO was verified by High Performance Liquid Chromatography (HPLC). According to the results of chromatogram, the retention time of probe MBI-CN was 14.95 min, and the retention time of MBI-CHO was 7.39 min. When the probe MBI-CN was placed in a hydrophobic environment, the results of chromatogram showed that a new chromatogram peak appeared at 7.40 min. This is basically consistent with the retention time of the chromatogram peak of MBI-CHO, which proves that the probe MBI-CN can undergo a hydrolysis reaction in a hydrophobic environment to generate MBI-CHO.

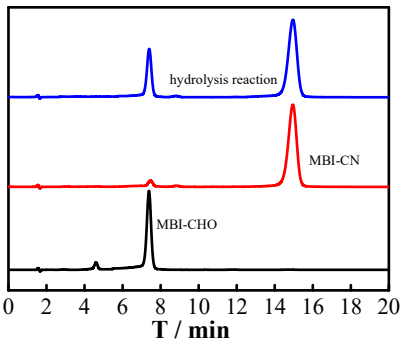


Fig. S3 The hydrolysis of MBI-CN to MBI-CHO in hydrophobic environment was verified by HPLC. (mobile phase: water/methanol (1:1, v/v), velocity of flow: 1.5 mL/min, ultraviolet detection wavelength: 380 nm).

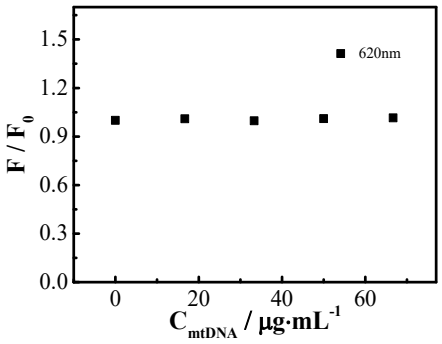


Fig. S4 Fluorescence response after adding mtDNA to MBI-CN in water. (F_0: the initial fluorescence intensity of probes MBI-CN; F: the fluorescence intensity after adding mtDNA).

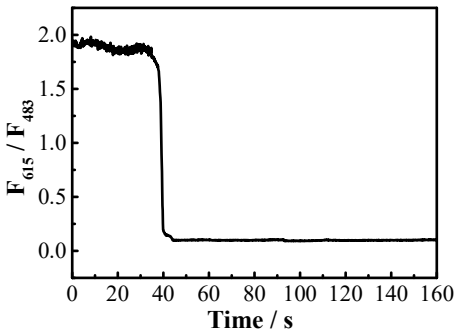


Fig. S5 Hydrolysis kinetics of MBI-CN.

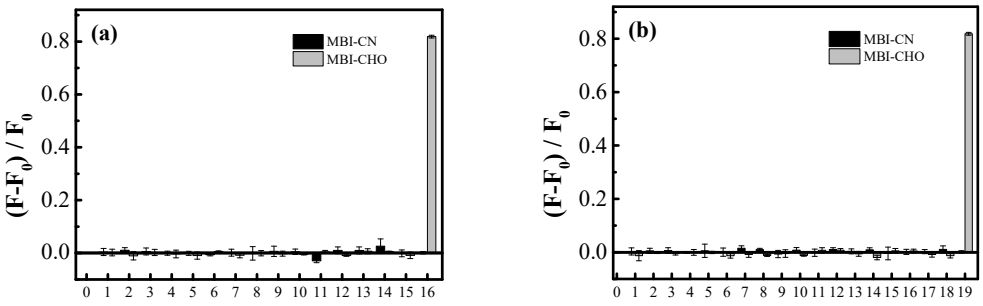


Fig. S6 Determination of MBI-CHO interference with related active small molecules in organisms. (a) Ion interference determination. 0 Blank;1 $SnCl_2$;2 $CaCl_2$;3 $MnCl_2$;4 $CoCl_2$;5 $CaSO_4$;6 $HgCl_2$;7 $Zn(NO_3)_2 \cdot 6H_2O$;8 $FeCl_2 \cdot 7H_2O$;9 $CaCl_2 \cdot 6H_2O$;10 $FeCl_3 \cdot 6H_2O$;11 $Al(NO_3)_3$;12 $MgSO_4$;13 Li_2CO_3;14 Na_2CO_3; 15 dipotassium hydrogen phosphate (20 μmol/L); 16 mtDNA (100 μg/mL). (b) Determination of amino acid interference. 0 Blank; 1 glycyl-DL-phenylalanine; 2 lysine; 3 DL-threonine; 4 cystine; 5 glutamine; 6 glutamic acid; 7 D-methionine; 8 L-cysteine; 9 glycine; 10 DL-leucine; 11 L-glutamic acid; 12 arginine; 13 hypoxanthine; 14 L-aspartic acid; 15 valine; 16 DL -Homocysteine; 17 Serine; 18 Glutathione (20 μmol/L); 19 mtDNA (100 μg/mL). (F_0: the initial fluorescence intensity of probes MBI-CN or MBI-CHO; F: the fluorescence intensity after adding interference).

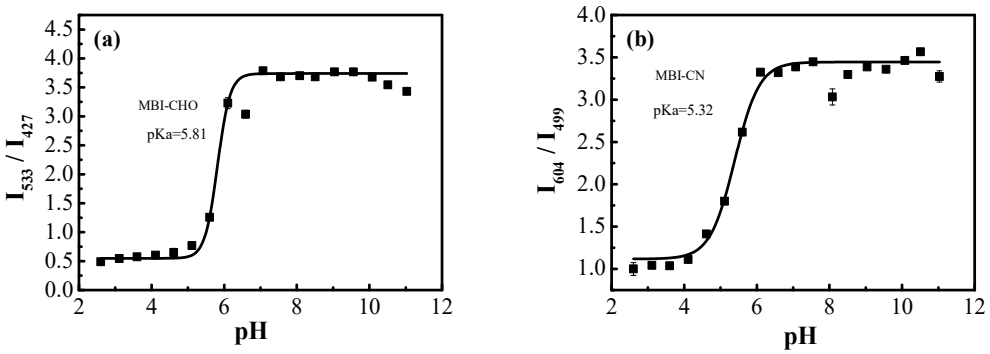


Fig. S7 Fluorescence signal changes of MBI-CHO and MBI-CN at different pH.

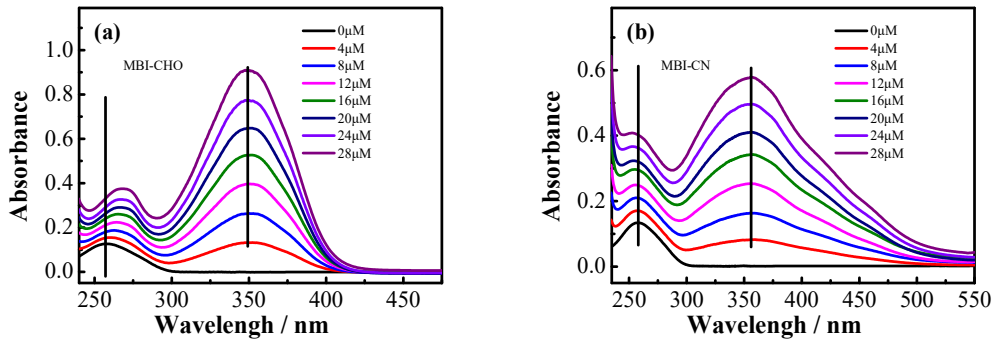


Fig. S8 In the Tris-HCl (0.05 mol, pH = 7.4), the concentration of fixed mtDNA (5 μg/mL) increased the concentration of the molecule (0-28 μmol/L). The interaction of

MBI-CHO and MBI-CN with mtDNA were tested by ultraviolet absorption spectroscopy

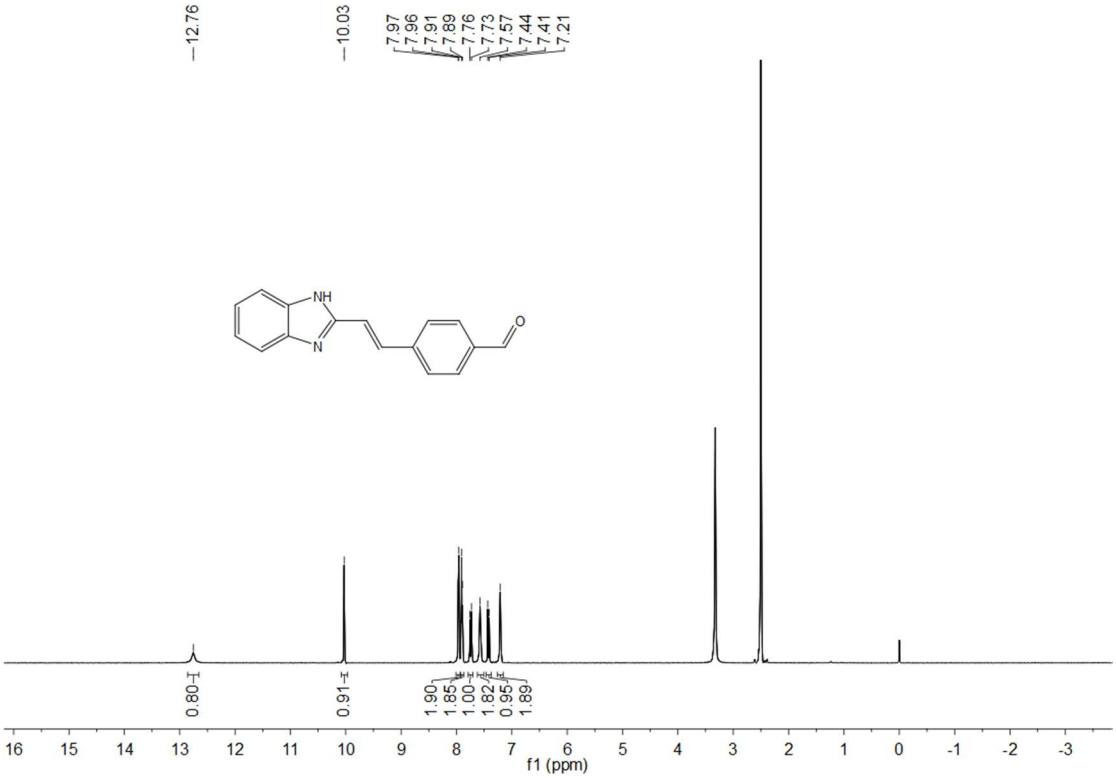


Fig. S9 ^{1}H NMR of compound MBI-CHO

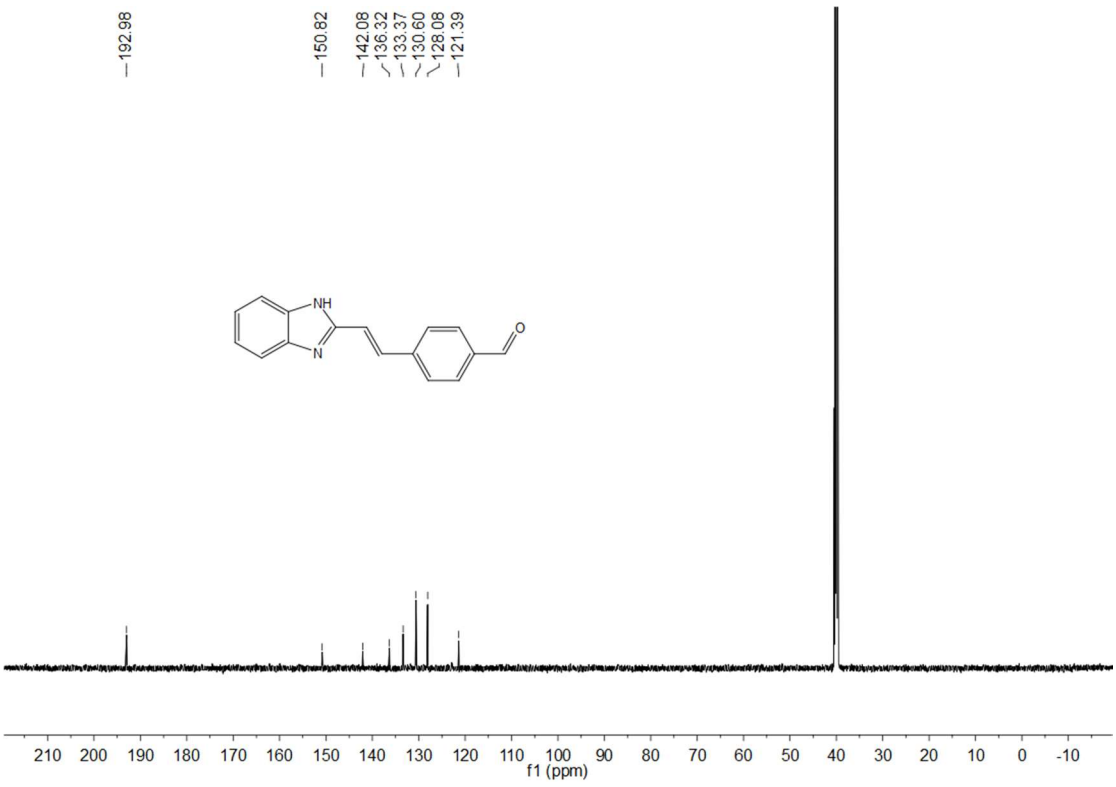
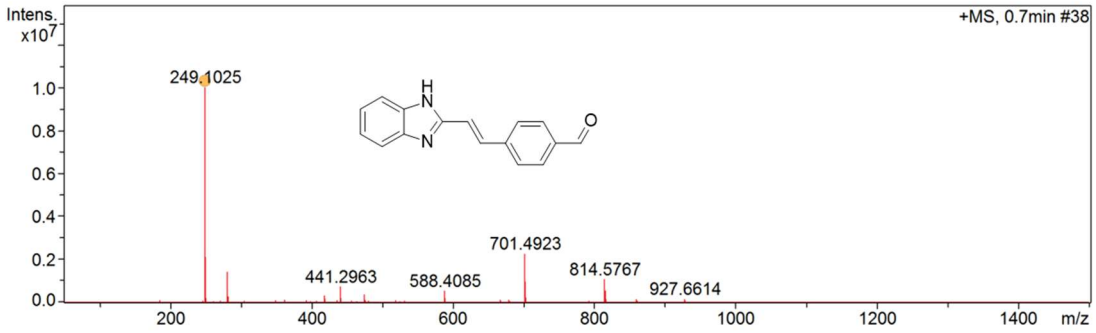


Fig. S10 ^{13}C NMR of compound MBI-CHO



Meas. m/z # Ion Formula m/z err [ppm] mSigma # mSigma Score rdb e¡¥ Conf N-Rule
249.1025 1 C16H13N2O 249.1022 -0.9 18.0 1 100.00 12.0 even ok

Fig. S11 Mass spectroscopy of compound MBI-CHO

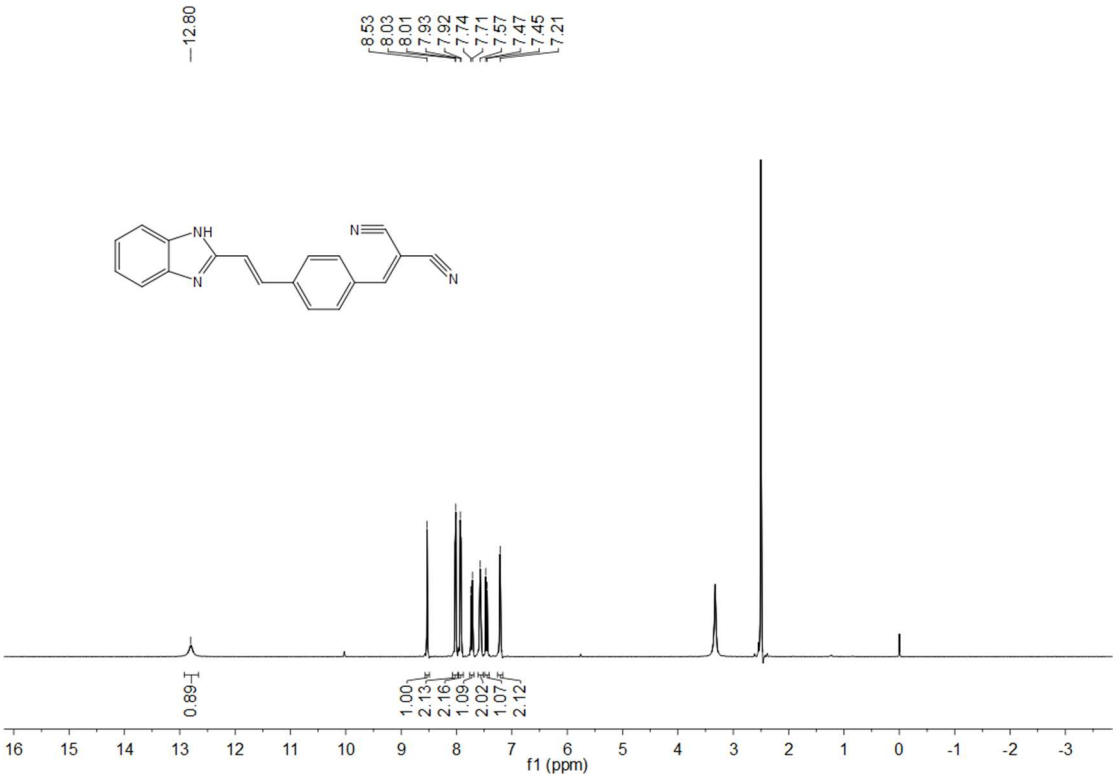


Fig. S12 ^{1}H NMR of compound MBI-CN

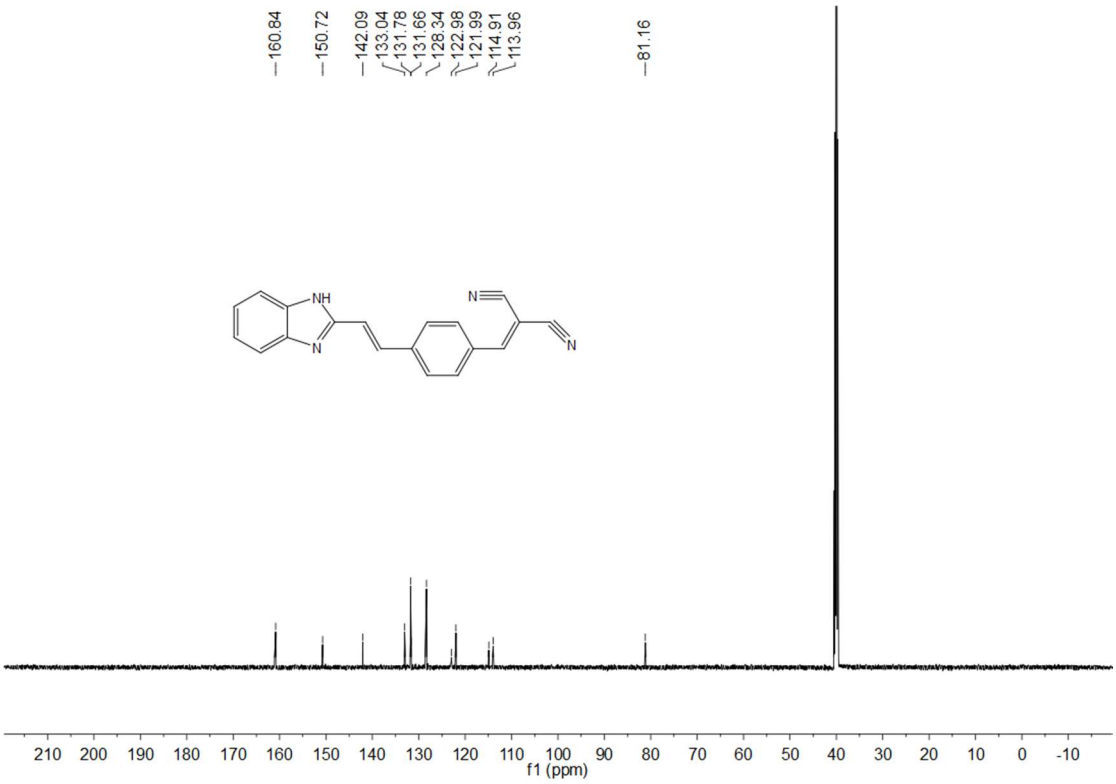
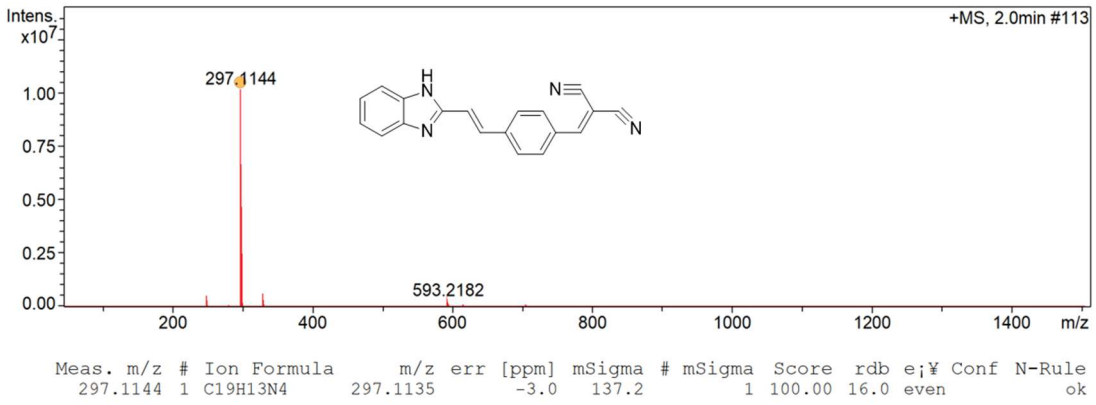


Fig. S13 ^{13}C NMR of compound MBI-CN



Meas. m/z # Ion Formula m/z err [ppm] mSigma # mSigma Score rdb e¡¥ Conf N-Rule
297.1144 1 C19H13N4 297.1135 -3.0 137.2 1 100.00 16.0 even ok

Fig. S14 Mass spectroscopy of compound MBI-CN