Supporting Information for

Nitroreductase and acidity dual functional ratiometric fluorescent probe for selectively imaging tumor cell

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HPLC analysis

HPLC was performed on SHIMADU CTO10AS with an AD-H column. The HPLC solvents employed were 20% isopropanol and 80% *n*-hexane. HPLC conditions were as follows: flow rate 1 mL/min, detection by UV (254 nm).

The reaction solution of **NAFP** (10 μM) and NADH (5 mM), nitroreductase (10 μg/mL) which was incubated in PBS buffer (0.01 M PBS, pH =7.0, contains 1% DMSO).

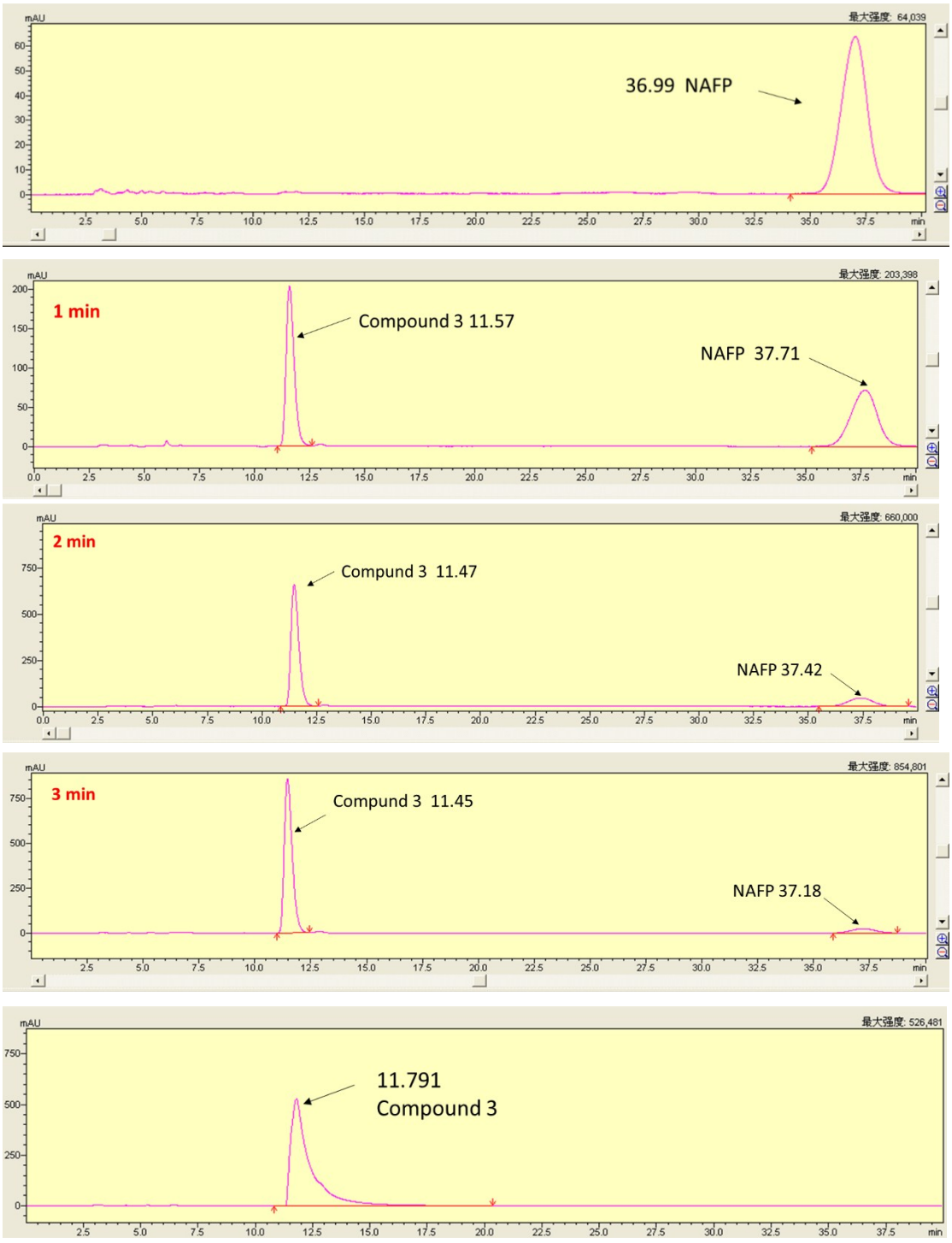


Figure S1. HPLC profiles a) **NAFP**; b) 1 min reaction solution; c) 2 min reaction solution; d) 3 min reaction solution; e) compound **3**.

LOD measurement:

The limit of detection (LOD) was calculated by IUPAC assay. LOD = 3Sb/m (Sb is the ration signal and noise, m is the slope of linear eqution). The standard deviation Sb is 0.14. According the linear equation of the fluorescence to the nitroreductase concentration at low concentration (following figure), the slope is 0.454×10^6. Hence, the detection limit is 0.92 μg/mL.

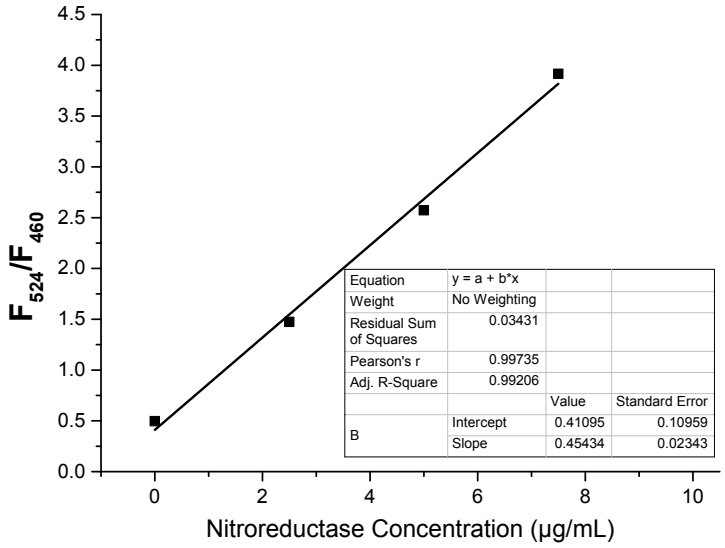
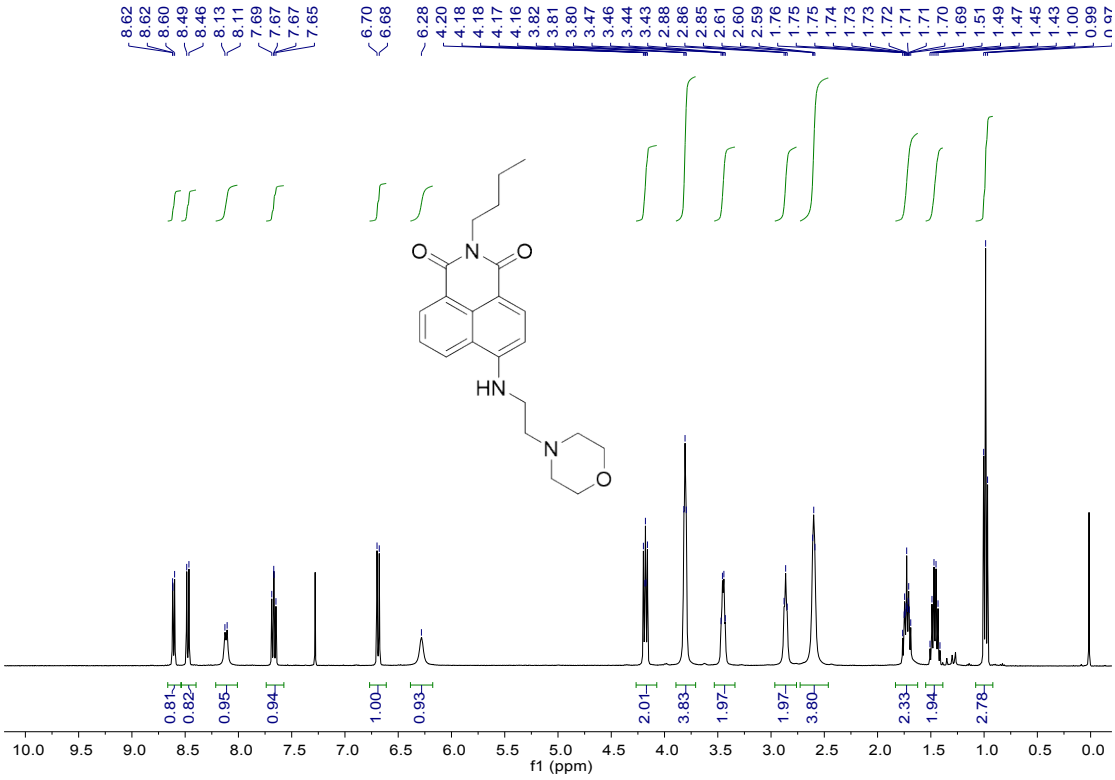
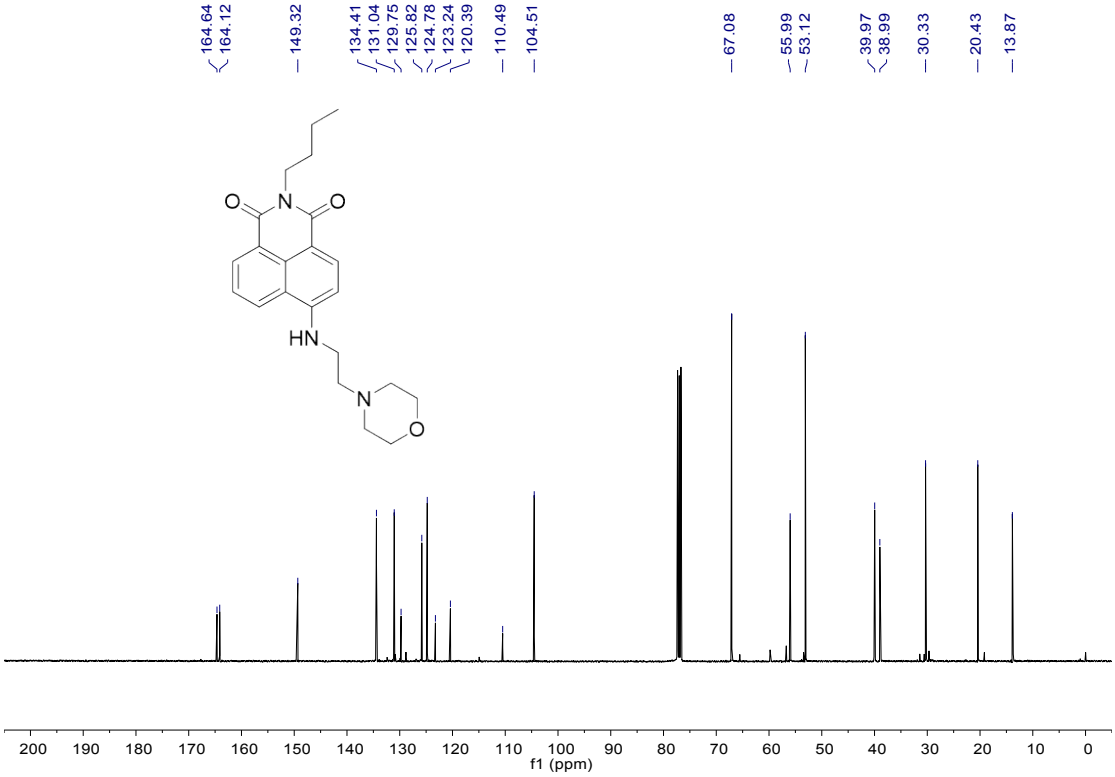
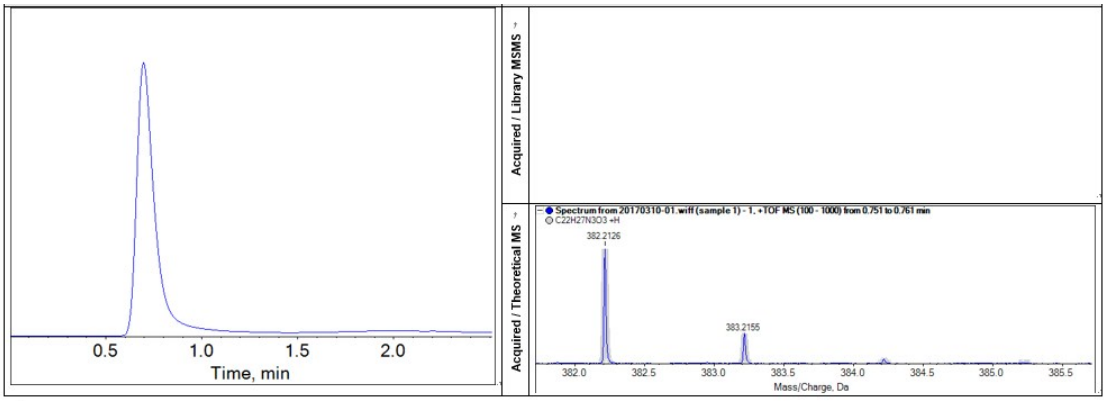


Figure S2. Emission ratio (F_{524}/F_{460}) of **NAFP** (10 μM) versus increasing concentration of nitroreductase in PBS buffer (1% DMSO, pH 5.0) in the presence of 500 μM of NADH with the excitation at 389 nm at 37 °C.

Characterization of compound **3**.

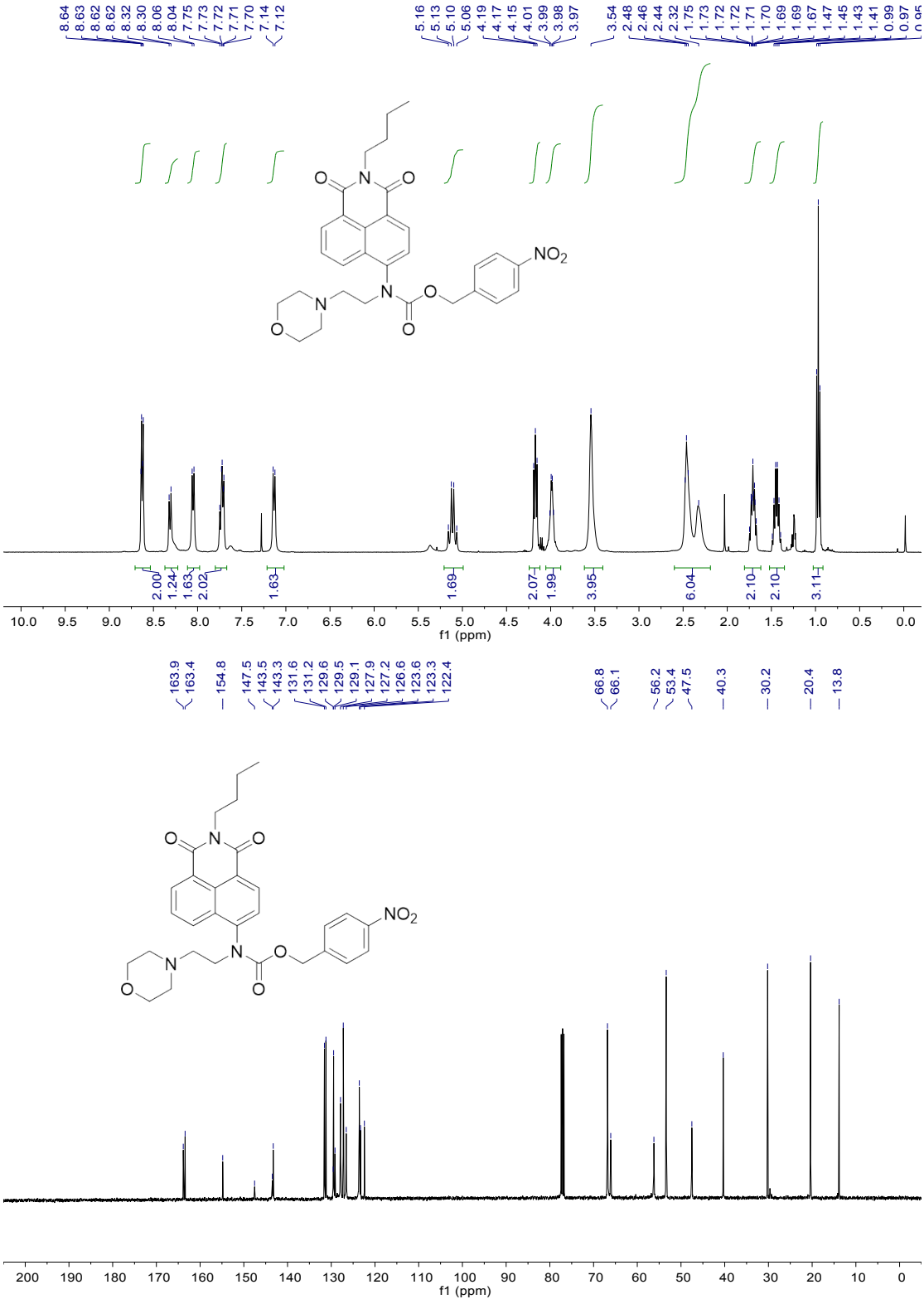


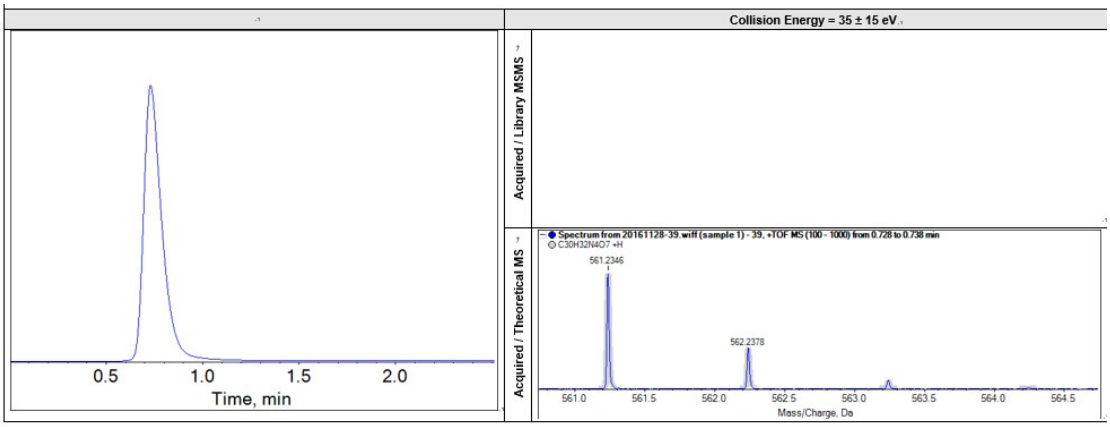




	Compound Name (Library Hit)	Score	Formula	Intensity	Threshold	Expected m/z	Found at m/z	Error (ppm)	Expected RT (min)	Found RT (min)	RT Delta (min)	Isotope Diff (%)	Library Score(%)
✓ ● ✓ ● ●	381.205242024	99%	C22H27N3O3	2163193	50	382.2125	382.2126	0.2	0.00	0.70	0.70	0.3%	N/A

Characterization of **NAFP**.





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✓ ● ✓ ● ●	560.227099792	97%	C30H32N4O7	2832655	50	561.2344	561.2346	0.4	0.00	0.73	0.73	0.4%	N/A