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Quantitative and Systematical Designing Fluorophores Enables Ultrasensitive Distinguishing Carbonyls

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Data Availability.

The authors declare that the main data supporting the findings of this study are available within the article and its Supplementary Information files. Extra data are available from the corresponding author upon request.

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Characterization of fluorescent reagent

High-resolution mass spectra (HRMS) were obtained on LCT Premier XE spectrometer, ESI-TOF (Waters, United States). The ^{1}H and ^{13}C NMR spectra were recorded on an AM 400 spectrometer, using TMS as an internal standard (Bruker United States) (Supplementary figure Fig. S4). Yield of **NH-4**: 85%; orange solid; mp 141–144 °C; FTIR (neat, v/cm^{-1}): 3438 (active hydrogen, N—H), 3321 (naphthalimide ring, C—H), 2971(tertiary carbon, C—H), 1682 (C═O), 1642, 1576, 1539 (C═C, naphthalimide ring), 1357, 1395 (C═C, naphthalimide ring), 1246 (C—N), 774 (in naphthalimide plane, C═C), 750 (δ, N—H);^{1}HNMR (100 MHz, CDCl$_3$) δ: 1.58 (6H, s, 2×CH$_3$), 3.84 (4H, s, NH$_2$), 5.41–5.48 (H, t, J = 7.0 Hz, CH), 6.56 (H, s, 2H, NH), 7.22–7.24 (H, d, J = 6.6 Hz, CH-5'), 7.62–7.65 (H, t, J = 6.6 Hz, CH-6'), 8.00–8.03 (H, d, J = 6.6 Hz, CH-3'), 8.51–8.53 (H, d, J = 6.6 Hz, CH-2'), 8.56–8.52 (H, d, J = 6.6 Hz, CH-8'). ESI-MS = 268 (M - H); HRMS *Calculated. for* C$_{15}$H$_{14}$N$_3$O$_2$: 268.1086. Found: 268.1075.

Absorption and fluorescence measurement

The samples for absorption spectra were prepared by successively placing acetonitrile (ACN, 850 μL), H$_2$O (950 μL) and one of **NH-1~7** (200 μL, 0.5 mM) in 3 mL cuvettes and were tested in a Varian Cary 500 UV/VIS spectrophotometer (Agilent, United States). Fluorescent measurements of the **NH-1~7-formaldehyde** were similar by placing acetonitrile (ACN, 750 μL), H2O (950 μL), one of **NH-1~7** (200 μL, 0.5 mM) and formaldehyde stock solution (1.0 mM, 100 μL) to obtain the final concentration of formaldehyde and fluorescent reagents both 0.05 mM in the 3

mL cuvettes. The slit width was set at 2.5 nm for excitation of 430 nm with photon multiplier voltage at 580 V on a Varian Cary Eclipse fluorescence spectrophotometer (Agilent, United States). The relative fluorescence quantum yield (Φf) was obtained by integrating the emission spectra of **NH-1~7-formaldehyde** to compare with Rhodamine 6G (Sigma-Aldrich, United States) and each sample was triply tested. The photography for fluorescent emission of sample vials (showed in Fig. 3a) were captured by a Canon EOS 70d digital SLR camera (Canon, Japan) with f/5.6, s-1.6 s, iso-100 at 5800 K (Shade) and f/5.6, s-1/20 s, iso-100 at 5800 K (Tint).

Preparation of UHPLC-FLD work solution

10 kinds of carbonyl species were involved in the UHPLC-FLD method development and validation., i.e. formaldehyde aqueous solution (37%) and acetaldehyde aqueous solution (40%) (Shanghai Chemicals, China), propylaldehyde (>95.0%), butyraldehyde (>98.0%), valeraldehyde (>98.0%), hexaldehyde (>98.0%), acetone (>99.5%), 2-Butanone (>99.0%), benzaldehyde (>98.0%) and m-methylbenzaldehyde (>97.0%) (Sigma-Aldrich, United States). The carbonyl solutions (0.01 mM - 0.001 nM) were diluted with initial mobile phase (ACN: water = 65:35, v/v) from standard stock solution (0.1 M). The fluorescent derivatization processes between carbonyls and **NH-4** were carried out at 25 °C for 15 min in 1.5 mL vials before testing. Briefly, 800 μL initial mobile phase was added to a vail, 100 μL of **NH-4** stock solution (0.5 mM) and 100 μL dilution solution of carbonyl was added in succession, 15 min later at 25 °C the vail was placed into UHPLC system for analysis.

Figures
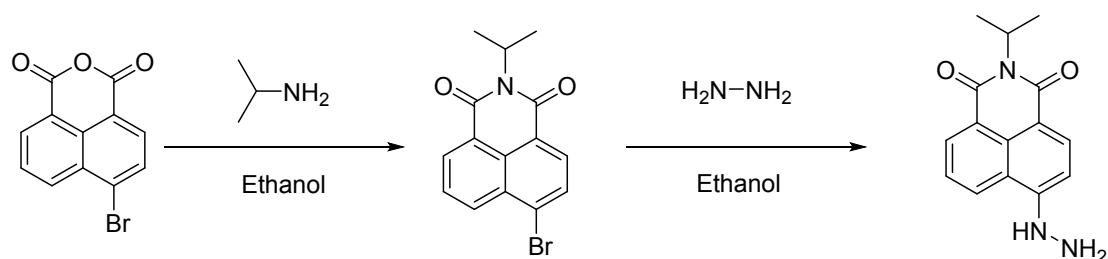
Fig. S1 Synthetic route of NH-4.
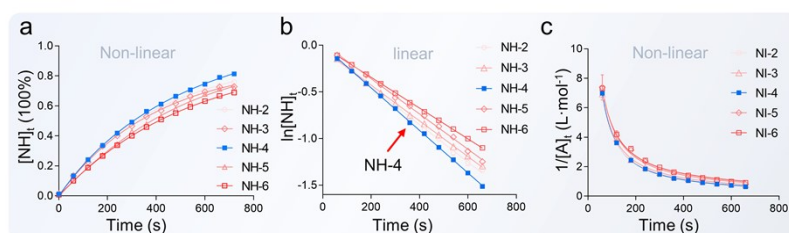
Fig. S2 The kinetic fitting curves of **NH-2~6** and formaldehyde, zero(a), first(b) and

second(c) order. Note: the first order fitting curves were showing the best linearity.
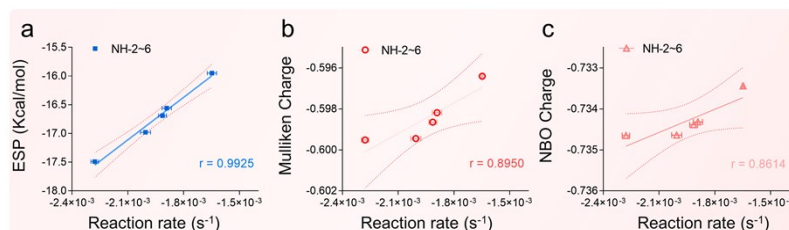
Fig. S3 The plots of rate constants k_{obs} and the negative charge population with

different theoretical calculation methods Electro-Static Potential (a), Mulliken charge

(b) and Natural bonding orbital (c).

Fig. S4 The ^{1}H NMR spectra of **NH-1~7** (tetramethylsilane, TMS as the internal standard, DMSO-d_6).
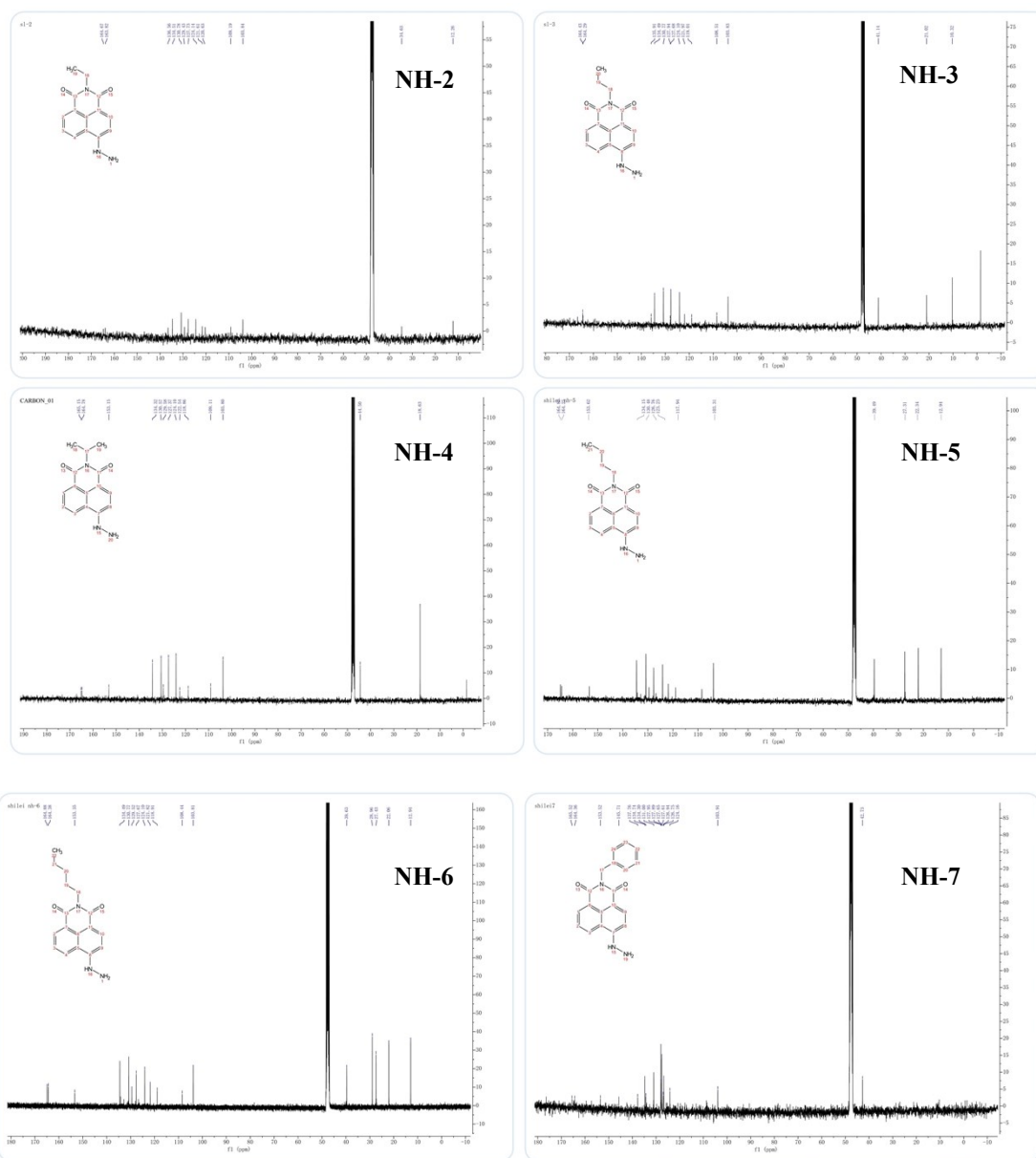
Fig. S5 The ^{13}C NMR spectra of **NH-2~7** (number of Scans 10000, tetramethylsilane, TMS as the internal standard, Methanol-d_4).

Tables

Table S1 LUMO energies of **NH-1~7** with DFT, B3-LYP/6-311G*

LUMO Energy (eV)	NH-1 (Methyl)	NH-2 (Ethyl)	NH-3 (n-Propyl)	NH-4 (i-Propyl)	NH-5 (Butyl)	NH-6 (Pentyl)	NH-7 (Benzyl)
Rea. Fluor. [a]	-2.454	-2.447	-2.446	-2.440	-2.445	-2.448	-2.487
Rea. Hydraz.	2.612	2.608	2.607	2.608	2.610	2.615	2.614
Prod. Fluor.	-2.438	-2.433	-2.432	-2.426	-2.429	-2.429	-2.469
Prod. Hydraz. [b]	-0.223	-0.223	-0.224	-0.223	-0.224	-0.226	-0.224

[a, b] "Rea." and "Prod." are representing the derivative reagents NH-1~7 and derivative products NH-1-formaldehyde ~ NH-7-formaldehyde. "Fluor." and "Hydraz." are representing the fluorescent naphthalimide moiety and hydrazine/ hydrazone moiety.

Table S2 HOMO energies of **NH-1~7** with DFT, B3-LYP/6-311G*

HOMO Energy (eV)	NH-1 (Methyl)	NH-2 (Ethyl)	NH-3 (n-Propyl)	NH-4 (i-Propyl)	NH-5 (Butyl)	NH-6 (Pentyl)	NH-7 (Benzyl)
Rea. Fluor.	-6.373	-6.368	-6.365	-6.355	-6.363	-6.362	-6.387
Rea. Hydraz.	-6.164	-6.181	-6.183	-6.178	-6.176	-6.148	-6.151
Prod. Fluor.	-6.376	-6.37	-6.367	-6.357	-6.365	-6.371	-6.375
Prod. Hydraz.	-7.014	-7.016	-7.013	-7.017	-7.014	-7.013	-7.013
ΔE [c]	0.638	0.647	0.645	0.660	0.649	0.642	0.638
Φ_f	65.97	78.31	77.25	88.04	76.13	72.00	67.27

[c] ΔE is the energy gap of products (NH-x-formaldehyde) between fluorescent naphthalimide moiety and hydrazone moiety.

Table S3 The C···C distances of naphthalimide cyclic [d]

Number	NH-1 (Methyl)	NH-2 (Ethyl)	NH-3 (n-Propyl)	NH-4 (i-Propyl)	NH-5 (Butyl)	NH-6 (Pentyl)	NH-7 (Benzyl)
1	3.8822	3.8815	3.8883	3.9133	3.8990	3.9077	3.8862
2	3.8806	3.8923	3.9032	3.9424	3.9151	3.9204	3.8845
3	3.7912	3.8053	3.8152	3.8667	3.8174	3.8157	3.7998
4	3.7577	3.7602	3.7636	3.7981	3.7612	3.7666	3.7524
4a	3.8522	3.8522	3.8576	3.8740	3.8669	3.8755	3.8583
5	3.9038	3.9104	3.9139	3.9706	3.9199	3.9491	3.9225
6	3.9121	3.9234	3.9388	3.9929	3.9481	3.9751	3.9612
7	3.8183	3.8172	3.8364	3.8629	3.8393	3.8502	3.8834
8	3.7596	3.7600	3.7622	3.7687	3.7519	3.7661	3.8044
8a	3.8518	3.8594	3.8491	3.8592	3.8540	3.8515	3.8624
Average	3.8410	3.8462	3.8528	3.8849	3.8573	3.8678	3.8615

[d] DFT calculation at B3-LYP/6-31++G** level.

Table S4 The rate constants k_{obs} of **NH-1~6** under pseudo-first order equation [e]

kinetic data (s^{-1})	NH-2 (Ethyl)	NH-3 (n-Propyl)	NH-4 (i-Propyl)	NH-5 (Butyl)	NH-6 (Pentyl)
k_{obs}, (283 K)	1.070×10^{-3}	0.985×10^{-3}	1.164×10^{-3}	0.970×10^{-3}	0.812×10^{-3}
R^2	0.9989	0.9996	0.9971	0.9996	0.9965
k_{obs}, (296 K)	2.005×10^{-3}	1.914×10^{-3}	2.278×10^{-3}	1.891×10^{-3}	1.648×10^{-3}
R^2	0.9983	0.9989	0.9998	0.9984	0.9998
k_{obs}, (313 K)	3.825×10^{-3}	3.566×10^{-3}	4.229×10^{-3}	3.500×10^{-3}	3.064×10^{-3}
R^2	0.9996	0.9999	0.9990	0.9991	0.9997

[e] All these kinetics data were obtained at target temperature ±0.5 K and repeated 5 times.

Table S5 Selected geometrical data of structures in the reaction of hydrazine NH-4 and formaldehyde with H_3O^+, H_2O and H^+ at B3-LYP/6-311+G**.

Transition state	distances/Å				Bond angle/degree			
	N^2-C	C-O^1	O^1-H	H-N^2	H-N^2-C	N^2-C-O^1	C-O^1-H	O^2-H-N^2
Trans. 1 (H_3O^+)	1.460	1.453	1.420	1.896	101.7	110.5	108.3	135.1
Trans. 1 (H_2O)	1.547	1.381	1.170	1.078	101.4	106.8	103.5	148.3
Trans. 1 (H^+)	1.474	1.485	1.207	1.383	73.9	94.1	78.6	113.3
Trans. 2 (H_3O^+)	1.291	2.307	0.987	-	-	114.1	112.5	-
Trans. 2 (H_2O)	1.319	2.009	1.112	1.151	108.6	107.8	88.8	144.7
Trans. 2 (H^+)	1.284	3.059	-	-	-	106.7	177.3	-

Table S6 Taking the **NH-4** with formaldehyde as an example to verify the delta Gibbs free energy ΔG (kcal·mol^{-1}), DFT TS calculation at B3-LYP/6-311+G**

Gibbs free energy	Reactant	TS1	IM	TS2	Product	Eyring function
H_3O^+	0	23.44	-0.75	12.74	-8.79	
H_2O	0	9.03	-9.09	25.28	-24.18	-7.88
H^+	0	40.02	-5.01	-5.13	-8.62	