Electronic Supplementary Information (ESI†)

Naphthalimide–coumarin conjugate: ratiometric fluorescent receptor for self-calibrating quantification of cyanide anion in cells

Yasuhiro Shiraishi, a,b* Naoto Hayashi, a Masaki Nakahata, c Shinji Sakai c and Takayuki Hirai a

a Research Center for Solar Energy Chemistry, and Division of Chemical Engineering, Graduate School of Engineering Science, Osaka University, Toyonaka 560-8531, Japan
b PRESTO, JST, Saitama 332-0012, Japan
c Division of Chemical Engineering, Graduate School of Engineering Science, Osaka University, Toyonaka 560-8531, Japan

shiraish@cheng.es.osaka-u.ac.jp

CONTENTS

| Table/FIG. | Description                                                                 | Page |
|-----------|-----------------------------------------------------------------------------|------|
| Table S1  | TD-DFT calculation results for 1′ and [1′–HCN]−                           | 2    |
| Fig. S1   | 1H NMR chart of 1                                                          | 3    |
| Fig. S2   | 13C NMR chart of 1                                                          | 4    |
| Fig. S3   | FAB-MS chart of 1                                                          | 5    |
| Fig. S4   | Time-dependent change in fluorescence spectra of 1                         | 6    |
| Fig. S5   | pH-Dependent change in the fluorescence intensity of 1                     | 7    |
| Fig. S6   | 1H–1H COSY chart of 1                                                      | 8    |
| Fig. S7   | 1H–1H COSY chart of [1′–HCN]−                                             | 9    |
| Fig. S8   | FAB-MS chart for the product obtained by the reaction of 1 with CN−        | 10   |
| Fig. S9   | Absorption spectra of 1 and [1′–HCN]−                                      | 11   |
| Fig. S10  | Cell viability                                                             | 11   |
| Cart. x   | Cartesian coordinates for respective compounds                              | 12   |
Table S1. Calculated excitation energy (E), wavelength (λ), and oscillator strength (f) for low-lying singlet state (Sn) of 1′ and [1′–HCN].

| Compound | Main Orbital Transition (CIC\(^a\)) | E (eV) [λ (nm)] | f |
|----------|---------------------------------------|-----------------|---|
| \(S_0 \rightarrow S_1\) | HOMO–1→LUMO (0.1274) HOMO→LUMO (0.66351) | 2.0822 eV [595.45 nm] | 0.2709 |
| \(S_0 \rightarrow S_2\) | HOMO–2→LUMO (–0.13519) HOMO–1→LUMO+2 (0.11064) | 2.4022 eV [516.12 nm] | 0.1303 |
| \(S_0 \rightarrow S_3\) | HOMO–1→LUMO (0.11524) HOMO–1→LUMO+2 (–0.118) HOMO→LUMO+1 (–0.1104) | 2.9877 eV [414.99 nm] | 0.3544 |
| \(S_0 \rightarrow S_4\) | HOMO–3→LUMO (0.68930) HOMO–2→LUMO+1 (0.54394) HOMO→LUMO+2 (0.3419) | 3.1157 eV [397.93 nm] | 0.1713 |
| \(S_0 \rightarrow S_5\) | HOMO–2→LUMO (0.59954) HOMO→LUMO+1 (0.12527) HOMO→LUMO+2 (–0.18121) | 3.2612 eV [380.17 nm] | 0.0172 |
| \(S_0 \rightarrow S_6\) | HOMO–3→LUMO (–0.18455) HOMO–2→LUMO+2 (–0.12133) | 3.3726 eV [367.62 nm] | 0.1713 |
| \(S_0 \rightarrow S_1\) | HOMO→LUMO (0.70568) | 2.2116 eV [560.61 nm] | 0.0003 |
| \([1′–HCN]\) | HOMO–1→LUMO (0.64000) HOMO–1→LUMO+3 (–0.11607) | 2.8779 eV [430.82 nm] | 0.3315 |
| \(S_0 \rightarrow S_3\) | HOMO–2→LUMO (0.68930) HOMO→LUMO+1 (–0.11225) | 3.2806 eV [377.93 nm] | 0.0067 |
| \(S_0 \rightarrow S_4\) | HOMO–2→LUMO (0.12200) HOMO→LUMO+1 (0.61102) | 3.4332 eV [361.13 nm] | 0.2942 |
| \(S_0 \rightarrow S_5\) | HOMO–3→LUMO (0.70271) | 3.6106 eV [343.39 nm] | 0.0021 |
| \(S_0 \rightarrow S_6\) | HOMO→LUMO+2 (0.70471) | 3.7164 eV [333.61 nm] | 0.0032 |

\(^a\) CI expansion coefficients for the main transitions.
Fig. S1  $^1$H NMR chart of 1 (10 mM, DMSO–d$_6$, 400 MHz).
Fig. S2  $^{13}$C NMR chart of 1 (30 mM, CDCl$_3$, 100 MHz).
Fig. S3  FAB-MS chart of 1.

[1 + H+]\(^+\) m/z 455.1601
(Calculated: [1 + H]\(^+\) m/z 455.1605)
Fig. S4  (a) Time-dependent change in fluorescence spectra of 1 (10 μM), measured with 40 μM of CN\(^{-}\) in a buffered water/MeCN mixture (1/1 v/v; HEPES 0.1 M, pH 7.0) at 25°C. (b) Change in the ratio of fluorescence intensity (Fl\(_{444}/\text{Fl}_{533}\)), where Fl\(_{444}\) is the intensity at \(\lambda_{\text{em}} = 444\) nm and Fl\(_{533}\) is the intensity at \(\lambda_{\text{em}} = 533\) nm, respectively. (c) Pseudo-first-order kinetic plot of the normalized fluorescence increase of 1 (10 μM) with 40 μM of CN\(^{-}\).
Fig. S5  pH-Dependent change in the fluorescence intensity at 444 nm and 533 nm of 1 (10 μM) measured in water/MeCN mixtures (1/1 v/v) at 25 °C with different pH (a) without and (b) with 200 equiv of CN⁻. (c) Change in the ratio of the fluorescence intensity (FI444/FI533) of 1 (10 μM) measured in water/MeCN mixtures (1/1 v/v) with different pH at 25 °C, (white) without and (black) with CN⁻.
Fig. S6 $^1$H-$^1$H COSY chart of 1 (30 mM, DMSO–d$_6$, 400 MHz). Colored circles indicate the observed cross peaks. The texts next to the circle mean the coupling protons.
Fig. S7 ¹H-¹H COSY chart of 1:1 association species for 1 and CN⁻ (30 mM, DMSO-d₆, 400 MHz). Colored circles indicate the observed cross peaks. The texts next to the circle mean the coupling protons.
Fig. S8
FAB–MS chart of 1:1 association species for I and CN-

[1' + HCN]– m/z 481.1638
(Calculated: [1' + HCN]– m/z 481.1638)
**Fig. S9** Change in the absorption spectra of **1** (10 μM) in a buffered water/MeCN mixture (1/1 v/v; HEPES 0.1 M, pH 7.0) at 25°C.

**Fig. S10** Viability of HeLa cells determined before and after 20 min incubation with DMF containing **1** (100 μM) at 37°C.
Cartesian Coordinates (in Å) of I’ (DFT/B3LYP/6-31+G*)

| Atom | C   | C   | C   | C   | C   | C   | C   | C   | C   | C   | C   | C   |
|------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
|      | 3.097334 | 2.92449 | -0.88103 | 1.772245 | 3.3787 | -0.99741 | 0.721157 | 2.601123 | -0.53239 | 0.963309 | 1.341692 | 0.06247 |
|      | 0.958726 | -0.82762 | 0.985532 | 4.98023 | 0.015239 | 0.485087 | 4.769491 | 1.2432 | -0.15856 | 5.27005 | 1.89259 | -0.58616 |
|      | 4.264314 | -1.89689 | 1.519495 | -1.39494 | 1.001802 | 0.388567 | -2.40941 | 0.210392 | 0.185319 | -3.78506 | 0.612631 | 0.152331 |
|      | -4.21684 | 2.01629 | 0.3249 | -5.65807 | 2.255645 | 0.218509 | -6.55799 | 1.257958 | -0.00461 | -6.15235 | -0.10798 | 0.16271 |
|      | -4.77186 | -0.37902 | -0.08078 | -7.05358 | -1.19378 | -0.39549 | -6.56864 | -2.47192 | -0.53521 | -5.16521 | -2.76508 | -0.45407 |
|      | -4.32796 | -1.66804 | -0.22366 | -8.54017 | -0.94394 | -0.48917 | -5.63299 | 1.50520 | 0.09538 | 5.16521 | 2.76508 | 0.45407 |
|      |     |     |     |     |     |     |     |     |     |     |     |     |

S12/S13
Cartesian Coordinates (in Å) of [1′−HCN]$^-$ (DFT/B3LYP/6–31+G*)

|   |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
|---|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| C | 3.208333 | 2.962385 | -0.46945 | O      | -2.88919 | 2.761697  | 0.332131 |
| C | 1.877622 | 3.404258 | -0.52087 | H      | 4.031708  | 3.622184  | -0.72497 |
| C | 0.840279 | 2.542783 | -0.19196 | H      | 1.654728  | 4.424806  | -0.81987 |
| C | 1.094    | 1.208945 | 0.20369  | H      | -0.17825  | 2.916087  | -0.24501 |
| C | 2.450855 | 0.758399 | 0.256073 | H      | -0.38903  | -1.72934  | 1.238273 |
| C | 3.495509 | 1.658612 | -0.08677 | H      | 1.95852   | -2.44742  | 1.280952 |
| C | 0.02828  | 0.280532 | 0.552424 | H      | -2.31684  | -1.11494  | 0.483674 |
| C | 0.381245 | -1.02604 | 0.943233 | H      | -5.1703   | 3.464648  | -0.68409 |
| C | 1.712684 | -1.43409 | 0.977378 | H      | -6.9812   | 1.919768  | -1.33534 |
| C | 2.757831 | -0.57356 | 0.642116 | H      | -7.40572  | -2.94597  | -1.16691 |
| C | 4.140175 | -1.04239 | 0.695939 | H      | -8.77565  | 0.263585  | -1.0282 |
| N | 5.145974 | -0.10123 | 0.362076 | H      | -8.07767  | 0.139346  | -2.64485 |
| C | 4.907242 | 1.21546  | -0.0427  | H      | -8.87516  | -1.26959  | -1.91682 |
| O | 5.846107 | 1.956792 | -0.34313 | H      | -1.5189   | 1.691219  | 0.383982 |
| O | 4.467222 | -2.18904 | 1.010999 | C      | -2.56786  | -0.16547  | 2.325124 |
| N | -1.25715 | 0.694915 | 0.465742 | N      | -2.67195  | -0.23624  | 3.48062 |
| C | -2.42784 | -0.08533 | 0.83878  | C      | 6.545761  | -0.55053  | 0.427047 |
| C | -3.68263 | 0.507245 | 0.205779 | H      | 6.598427  | -1.30861  | 1.210652 |
| C | -3.81492 | 1.936587 | 0.022947 | H      | 7.146299  | 0.315799  | 0.712642 |
| C | -5.06108 | 2.391696 | -0.55058 | C      | 7.046972  | -1.12789  | -0.90277 |
| C | -6.06183 | 1.523686 | -0.91023 | H      | 6.410147  | -1.97684  | -1.18415 |
| C | -5.92959 | 0.117825 | -0.74676 | H      | 6.942845  | -0.36455  | -1.685 |
| C | -4.71421 | -0.34311 | -0.17997 | C      | 8.50941   | -1.58438  | -0.81908 |
| C | -6.92132 | -0.84953 | -1.1052  | H      | 9.139157  | -0.73384  | -0.51891 |
| C | -6.6778  | -2.18602 | -0.90379 | H      | 8.61013   | -2.33889  | -0.0251 |
| C | -5.44607 | -2.66722 | -0.33623 | C      | 9.03303   | -2.16291  | -2.13941 |
| O | -4.5118  | -1.68882 | 0.017777 | H      | 10.07972  | -2.48098  | -2.05151 |
| C | -8.23408 | -0.40802 | -1.70646 | H      | 8.443457  | -3.03528  | -2.44975 |
| O | -5.13239 | -3.82868 | -0.13186 | H      | 8.975768  | -1.42051  | -2.94605 |