# Supporting Information

# Reactive Cyanine Fluorescence Dyes Indicating pH Perturbation of Biomolecules

**Jin Woo Park,[†,‡] Dong Jin Kim,[†] Kee-Jung Lee,[‡,*] and YoungSoo Kim[†,*]**

[†]*Center for Neuro-Medicine, Brain Science Institute, Korea Institute of Science and Technology, Seoul 136-791, Korea*
[*]*E-mail: yskim@bio.kist.re.kr*
[‡]*Department of Chemical Engineering, Hanyang University, Seoul 133-791, Korea.* [*]*E-mail: leekj@hanyang.ac.kr*
*Received September 19, 2012, Accepted October 22, 2012*

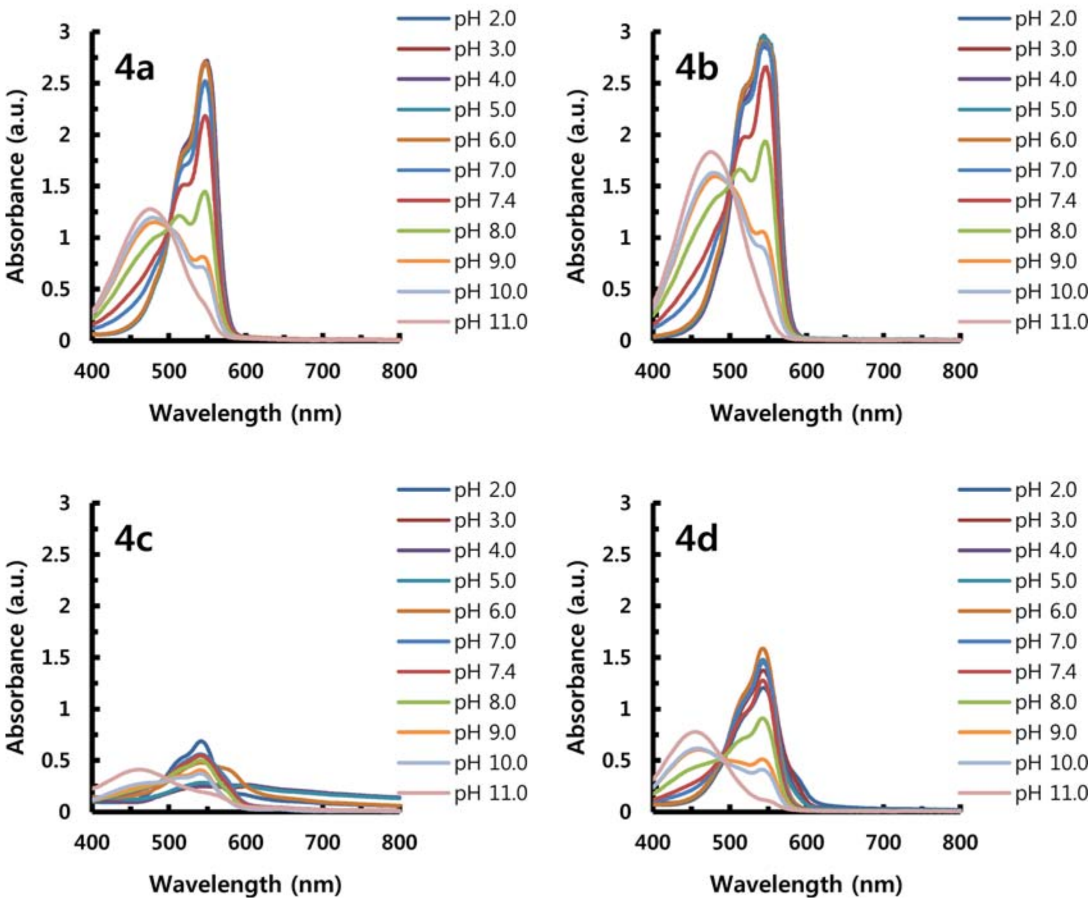



**Figure 1.** Variation of absorbance intensity of VS cyanine dyes over pH Range. Stock solutions of **4a-l** (10 mg/mL in DMF, 1 μL) was diluted with 0.1 M sodium phosphate buffers (pH 2, 3, 4, 5, 6, 7, 7.4, 8, 9, 10, and 11), and absorbance spectrum was measured by Enspire™ 2300 multilabel plate reader (Perkin Elmer, MA).

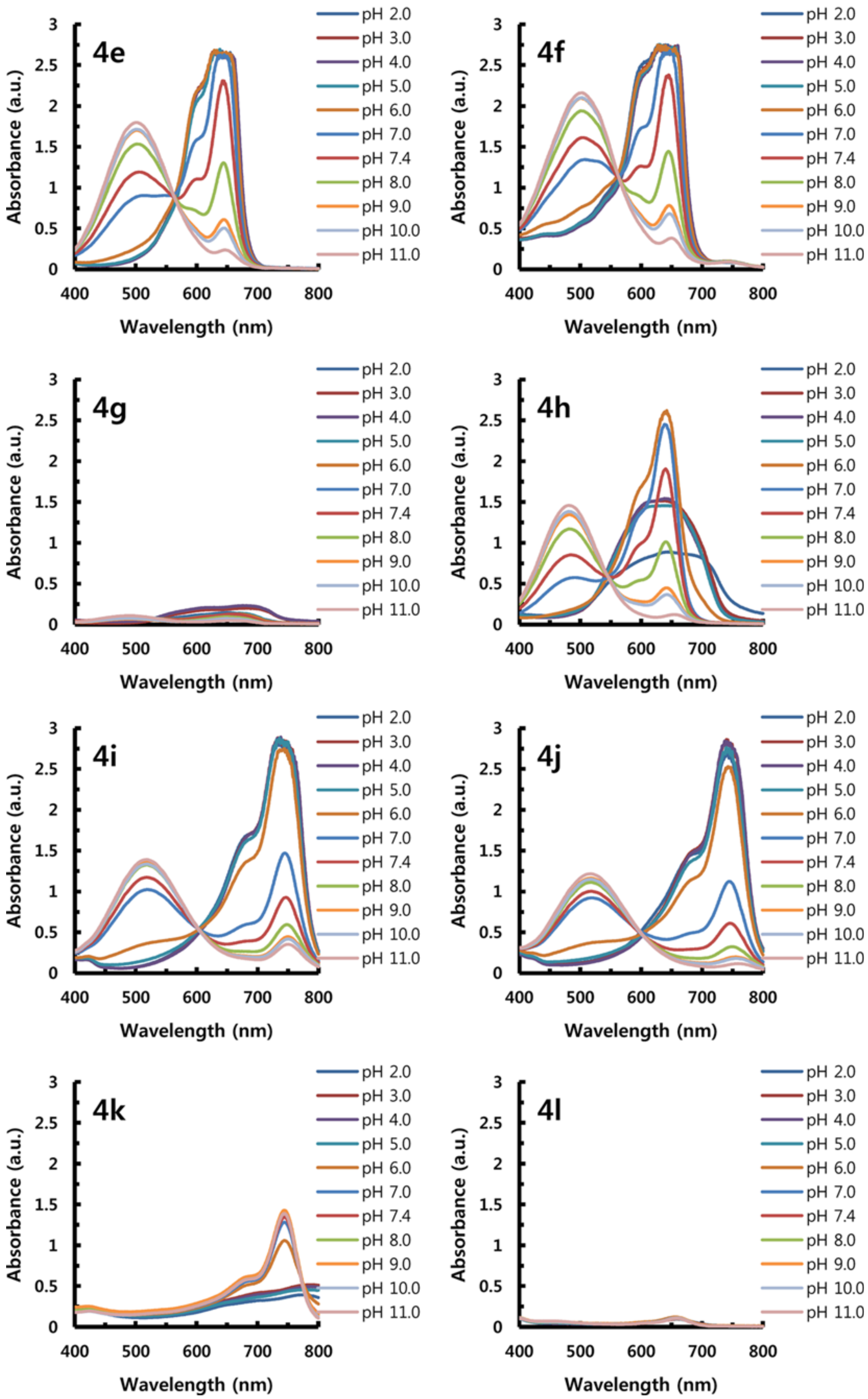
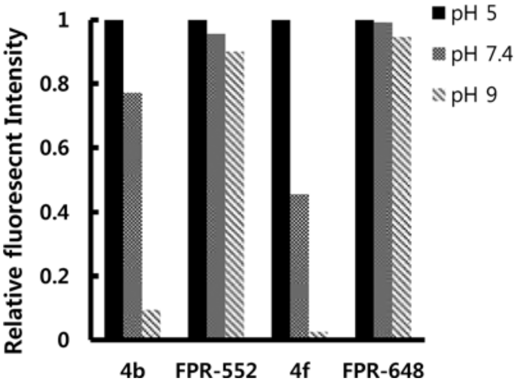
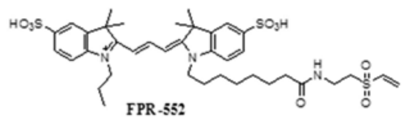
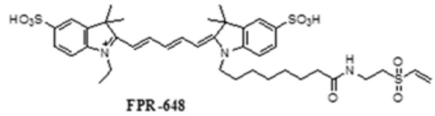



**Figure 2.** Emission intensity comparison of pH insensitive dyes (FPR-552, FPR-648) and pH sensive dyes (**4b**, **4f**). All dyes were dissolved in distilled water at 1 mg/100 μL, and were diluted to 0.0025 mM for measurement of fluorescence spectrum with LS-55 fluorescence spectrophotometer (Perkin Elmer, MA).





FPR-552


FPR-648

**Chemical data of VS Cyanine Dyes (4a-l)**

Synthesis of compound **1a-1f**, **2a-d**, **3a-l** and **4a-l** was followed in the literatures.[6,11]

2-[(1*E*,3*E*)-3-[3,3-dimethyl-1-[6-(2-(vinylsulfonyl)ethyl-amino)-6-oxohexyl]-5-sulfoindolin-2-ylidene]-1-propen-1-yl]-3,3-dimethyl-5-sulfo-3*H*-Indolium (**4a**); $^1$H NMR (500 MHz, DMSO-$d_6$) δ 8.22-8.13 (br m, 1H), 8.01 (t, 1H, $J$ = 5.5 Hz), 7.87 (s, 1H), 7.73 (s, 1H), 7.68-7.61 (m, 2H), 7.36-7.33 (m, 1H), 7.21 (d, 1H, $J$ = 8.1 Hz), 6.99 (q, 1H, $J$ = 11.1 Hz), 6.44-6.37 (br m, 1H), 6.27-6.16 (m, 3H), 4.17-4.08 (br m, 2H), 3.22 (t, 2H, $J$ = 9.4 Hz), 3.16-3.11 (m, 2H), 2.52 (t, 2H, $J$ = 7.3 Hz), 1.71-1.19 (br m, 18H); LC/MS *m/z* 712.2 ($C_{33}H_{40}N_3O_9S_3^-$ requires 718.19).

2-[(1*E*,3*E*)-3-[3,3-dimethyl-1-[8-(2-(vinylsulfonyl)ethyl-amino]-8-oxooctyl]-5-sulfoindolin-2-ylidene]-1-propen-1-yl]-3,3-dimethyl-5-sulfo-3*H*-Indolium (**4b**); $^1$H NMR (500 MHz, DMSO-$d_6$) δ 8.21-8.12 (br m, 1H), 7.95 (t, 1H, $J$ = 5.5 Hz), 7.87 (br s, 1H), 7.72 (s, 1H), 7.66-7.61 (m, 2H), 7.40-7.34 (m, 1H), 7.18 (d, 1H, $J$ = 8.5 Hz), 6.96 (q, 1H, $J$ = 13.2 Hz), 6.43-6.41 (m, 1H), 6.25 (s, 1H), 6.22 (d, 1H, $J$ = 5.5 Hz), 6.17 (d, 1H, $J$ = 13.5 Hz), 4.14-4.07 (br m, 2H), 3.22 (t, 2H, $J$ = 6.5 Hz), 3.17-3.11 (m, 2H), 2.02 (t, 2H, $J$ = 7.5 Hz), 1.71-1.21 (br m, 22H); LC/MS *m/z* 746.2 ($C_{35}H_{44}N_3O_9S_3^-$ requires 746.22).

2-[(1*E*,3*E*)-3-[3,3-dimethyl-1-[8-(2-(vinylsulfonyl)ethyl-amino)-8-oxooctyl]indolin-2-ylidene]-1-propen-1-yl]-3,3-dimethyl-3*H*-Indolium (**4c**); $^1$H NMR (500 MHz, $CDCl_3$-*d*) δ 7.95 (t, 1H, $J$ = 13.5 Hz), 7.64-7.61 (m, 1H), 7.28-7.11 (m, 8H), 6.89 (t, 1H, $J$ = 7.5 Hz), 6.68-6.62 (m, 2H), 6.48-6.41 (m, 1H), 6.22-6.19 (m, 1H), 6.99 (q, 1H, $J$ = 11.1 Hz), 6.44-6.37 (br m, 1H), 6.27-6.16 (m, 3H), 3.76-3.61 (br m, 2H), 3.20 (t, 2H, $J$ = 6.0 Hz), 3.15 (t, 2H, $J$ = 6.5 Hz), 2.22-2.18 (m, 2H), 1.73-0.86 (br m, 22H); LC/MS *m/z* 588.6 ($C_{35}H_{46}N_3O_3S^+$ requires 588.33).

2-[(1*E*,3*E*)-3-[3,3-dimethyl-1-[8-(2-(vinylsulfonyl)ethyl-amino)-8-oxooctyl]-5-sulfoindolin-2-ylidene]-1-propen-1-yl]-3,3-dimethyl-3*H*-Indolium (**4d**); $^1$H NMR (500 MHz, DMSO-$d_6$) δ 8.13-8.05 (m, 2H), 7.88-7.84 (m, 1H), 7.80-7.74 (m, 2H), 7.68-7.63 (m, 2H), 7.28-7.17 (m, 2H), 7.15-6.95 (m, 1H), 6.36-6.34 (m, 1H), 6.28-6.23 (m, 3H), 4.19-4.09 (m, 2H), 3.23-3.09 (m, 4H), 2.36-2.30 (m, 2H), 1.67-1.01 (br m, 22H); LC/MS *m/z* 668.34 ($C_{35}H_{46}N_3O_6S_2^+$ requires 668.28).

2-[(1*E*,3*E*)-5-[3,3-dimethyl-1-[6-(2-(vinylsulfonyl)ethyl-amino)-6-oxohexyl]-5-sulfoindolin-2-ylidene]-1,3-pentadi-en-1-yl]-3,3-dimethyl-5-sulfo-3*H*-Indolium (**4e**); $^1$H NMR (500 MHz, DMSO-$d_6$) δ 8.20-8.16 (m, 2H), 8.02-7.99 (m, 1H), 7.75 (d, 2H, $J$ = 4.3 Hz), 7.62 (q, 2H, $J$ = 10.6 Hz), 7.28 (d, 1H, $J$ = 7.7 Hz), 7.14 (d, 1H, $J$ = 8.0 Hz), 6.98 (q, 1H, $J$ = 13.2 Hz), 5.51 (t, 1H, $J$ = 12 Hz), 6.26-6.23 (m, 3H), 6.14 (d, 1H, $J$ = 13.8 Hz), 4.08-4.01 (br m, 2H), 3.35-3.33 (m, 2H), 3.23 (t, 2H, $J$ = 6.5 Hz), 2.07-2.03 (m, 2H), 1.68-1.32 (br m, 18H); LC/MS *m/z* 746.2 ($C_{35}H_{44}N_3O_9S_3^+$ requires 746.22).

2-[(1*E*,3*E*)-5-[3,3-dimethyl-1-[8-(2-(vinylsulfonyl)ethyl-amino]-8-oxooctyl]-5-sulfoindolin-2-ylidene]-1,3-pentadi-en-1-yl]-3,3-dimethyl-5-sulfo-3*H*-Indolium (**4f**); $^1$H NMR (500 MHz, DMSO-$d_6$) δ 8.20-8.14 (m, 2H), 7.95 (t, 1H, $J$ = 5.5 Hz), 7.73 (br s, 2H), 7.59-7.57 (m, 2H), 7.16-7.14 (m, 2H), 6.97 (q, 1H, $J$ = 13.2 Hz), 6.50-6.42 (m, 1H), 6.25-6.15 (br m, 4H), 4.06-3.97 (br m, 2H), 3.23 (t, 2H, $J$ = 7.0 Hz), 3.17-3.12 (m, 2H), 2.02 (t, 2H, $J$ = 7.0 Hz), 1.69-1.23 (br m, 22H); LC/MS *m/z* 771.8 ($C_{37}H_{46}N_3O_9S_3^-$ requires 772.24).

2-[(1*E*,3*E*)-5-[3,3-dimethyl-1-[8-(2-(vinylsulfonyl)ethyl-amino)-8-oxooctyl]indolin-2-ylidene]-1,3-pentadien-1-yl]-3,3-dimethyl-3*H*-Indolium (**4g**); $^1$H NMR (500 MHz, $CDCl_3$-*d*) δ 8.24 (br s, 2H), 8.12 (d, 1H, $J$ = 7.5 Hz), 7.72 (t, 2H, $J$ = 4.0 Hz), 7.66-7.46 (m, 6H), 7.20-7.15 (m, 1H), 6.67 (q, 3H, $J$ = 13.2 Hz), 6.45 (m, 1H), 6.22 (d, 1H, $J$ = 10.0 Hz), 4.47 (br s, 2H), 3.30-3.23 (m, 4H), 2.29-2.23 (m, 2H), 2.00-0.89 (br m, 22H); LC/MS *m/z* 614.0 ($C_{37}H_{48}N_3O_3S^+$ requires 614.34).

2-[(1*E*,3*E*)-5-[3,3-dimethyl-1-[8-(2-(vinylsulfonyl)ethyl-amino)-8-oxooctyl]-5-sulfoindolin-2-ylidene]-1,3-pentadi-en-1-yl]-3,3-dimethyl-3*H*-Indolium (**4h**); $^1$H NMR (500 MHz, DMSO-$d_6$) δ 8.20-8.01 (m, 3H), 7.76 (s, 1H), 7.62 (d, 1H, $J$ = 9.0 Hz), 7.55 (d, 1H, $J$ = 7.5 Hz), 7.34 (t, 1H, $J$ = 7.5 Hz), 7.25 (d, 1H, $J$ = 8.0 Hz), 7.21-7.17 (m, 2H), 7.03-7.00 (m, 1H), 6.51 (t, 1H, $J$ = 12.5 Hz), 6.34-6.27 (m, 3H), 6.16-6.13 (d, 1H, $J$ = 13.5 Hz), 4.05-4.03 (m, 2H), 3.11-3.36 (m, 4H), 2.18 (t, 2H, $J$ = 7.0 Hz), 1.70-1.23 (br m, 22H); LC/MS *m/z* 694.64 ($C_{37}H_{48}N_3O_6S_2^+$ requires 694.3).

2-[(1*E*,3*E*)-7-[3,3-dimethyl-1-[6-(2-(vinylsulfonyl)ethyl-amino)-6-oxohexyl]-5-sulfoindolin-2-ylidene]-1,3,5-hep-tatrien-1-yl]-3,3-dimethyl-5-sulfo-3*H*-Indolium (**4i**); $^1$H NMR (500 MHz, DMSO-$d_6$) δ 8.25-8.17 (br s, 1H), 8.06-7.96 (m, 2H), 7.90-7.52 (br m, 7H), 7.01-6.95 (m, 1H), 6.60-6.48 (m, 2H), 6.26-6.23 (m, 4H), 4.18-4.14 (m, 2H), 3.24-3.21 (m, 2H), 3.15-3.10 (m, 2H), 2.05-2.03 (m, 2H), 1.65-1.22 (br m, 18H); LC/MS *m/z* 770.3 ($C_{37}H_{44}N_3O_9S_3^-$ requires 770.22).

2-[(1*E*,3*E*)-7-[3,3-dimethyl-1-[8-(2-(vinylsulfonyl)ethylamino]-8-oxooctyl]-5-sulfoindolin-2-ylidene]-1,3,5-heptatrien-1-yl]-3,3-dimethyl-5-sulfo-3*H*-Indolium (**4j**); $^1$H NMR (500 MHz, DMSO-$d_6$) δ 8.21-8.12 (br s, 1H), 7.96 (t, 2H, *J* = 5.5 Hz), 7.90-7.88 (m, 1H), 7.77-7.75 (m, 2H), 7.65 (s, 2H), 7.56 (d, 2H, *J* = 9.0 Hz), 6.98 (q, 1H, *J* = 13.2 Hz), 6.46-6.37 (m, 2H), 6.27-6.23 (m, 4H), 3.98-3.91 (m, 2H), 3.28-3.21 (m, 2H), 3.17-3.12 (m, 2H), 2.03 (t, 2H, *J* = 7.5 Hz), 1.69-1.23 (br m, 22H); LC/MS *m/z* 797.7 ($C_{39}H_{48}N_3O_9S_3^-$ requires 798.26).

2-[(1*E*,3*E*)-7-[3,3-dimethyl-1-[8-(2-(vinylsulfonyl)ethylamino)-8-oxooctyl]indolin-2-ylidene]-1,3,5-heptatrien-1-yl]-3,3-dimethyl-3*H*-Indolium (**4k**); $^1$H NMR (500 MHz, $CDCl_3$-*d*) δ 8.39-8.37 (m, 1H), 8.08-7.92 (br m, 3H), 7.61-7.48 (br m, 8H), 6.98-6.96 (m, 2H), 6.84 (d, 2H, *J* = 7.5 Hz), 6.75-6.71 (m, 1H), 6.35 (m, 2H), 4.20-4.13 (br m, 2H), 3.31-3.29 (m, 2H), 3.24-3.22 (m, 2H), 2.03-2.00 (m, 2H), 1.70-0.83 (br m, 22H); LC/MS *m/z* 640.0 ($C_{39}H_{50}N_3O_3S^+$ requires 640.36).

2-[(1*E*,3*E*)-7-[3,3-dimethyl-1-[8-(2-(vinylsulfonyl)ethylamino)-8-oxooctyl]-5-sulfoindolin-2-ylidene]-1,3,5-heptatrien-1-yl]-3,3-dimethyl-3*H*-Indolium (**4l**); $^1$H NMR (500 MHz, DMSO-$d_6$) δ 8.32-8.14 (m, 3H), 8.04-7.99 (m, 1H), 7.92-7.41 (br m, 7H), 6.90-6.82 (m, 1H), 6.58-6.42 (m, 2H), 6.29-6.10 (m, 4H), 4.05-3.96 (m, 2H), 3.19-3.08 (m, 4H), 2.00-1.96 (m, 2H), 1.71-0.84 (br m, 22H); LC/MS *m/z* 720.54 ($C_{39}H_{50}N_3O_6S_2^+$ requires 720.31).