Supporting Information.

A Quick Responsive Fluorogenic pH Probe for Ovarian Tumor Imaging

Ching-Hsuan Tung*, Jianjun Qi, Lingchuan Hu, Myung Shin Han, and Young Kim

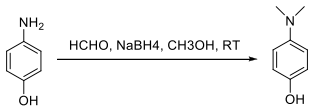
*Correspondence to:

Ching H. Tung, Weill Cornell Medical College, 413 East 69th Street, Box 290, New York, NY 10021. E-mail: cht2018@med.cornell.edu

1. Preparation of dye CypH-1
    a. Synthesis of 4-(dimethylamino)phenol
    b. Synthesis of CypH-1
    c. ^{1}HNMR spectra of 4-(dimethylamino)phenol
    d. Mass spectra of 4-(dimethylamino)phenol
    e. ^{1}HNMR spectra of dye CypH-1
    f. Mass spectra of dye CypH-1
    g. ^{13}CNMR spectra of dye CypH-1
2. Fig. S-1 Normalized absorption and emission spectra of dye CypH-1 in pH 4.0.
3. Fig. S-2 Quantum yield measurement of CypH-1.
4. Fig. S-3 LogP measurement of CypH-1

Preparation of dye CypH-1

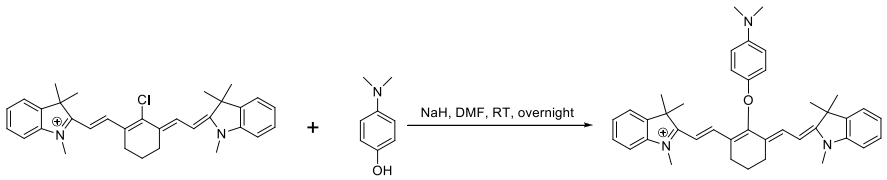
4-(dimethylamino)phenol



4-Aminophenol hydrochloride (1.33 g, 9.14 mmol) was dissolved in methanol/formaldehyde (30 ml/9 ml) at 0 ^{o}C. Sodium borohydride (3.50 g, 93 mmol) was added to the reaction solution slowly. The solution was stirred for one more hour, and then water (30 ml) was added. The reaction product was extracted with ethyl acetate (3 X), and washed with brine. The combined organic layers were dried over sodium sulfate. After concentration, the residue was purified by silica gel column, eluted with hexane and ethyl acetate (2/1) to give 780 mg of crystal solid, yield (62.4%). TLC: hexane/acetate=1/1, R_f=0.4. ^{1}H-NMR (300 MHz, MeOD): 6.80 (2H, d, J=6.9Hz), 6.71(2H, d, J=6.9Hz), 2.79 (6H, S). MS:138 (M^{+}+H).

Reference: JACS 2011, 133, 16970-6.

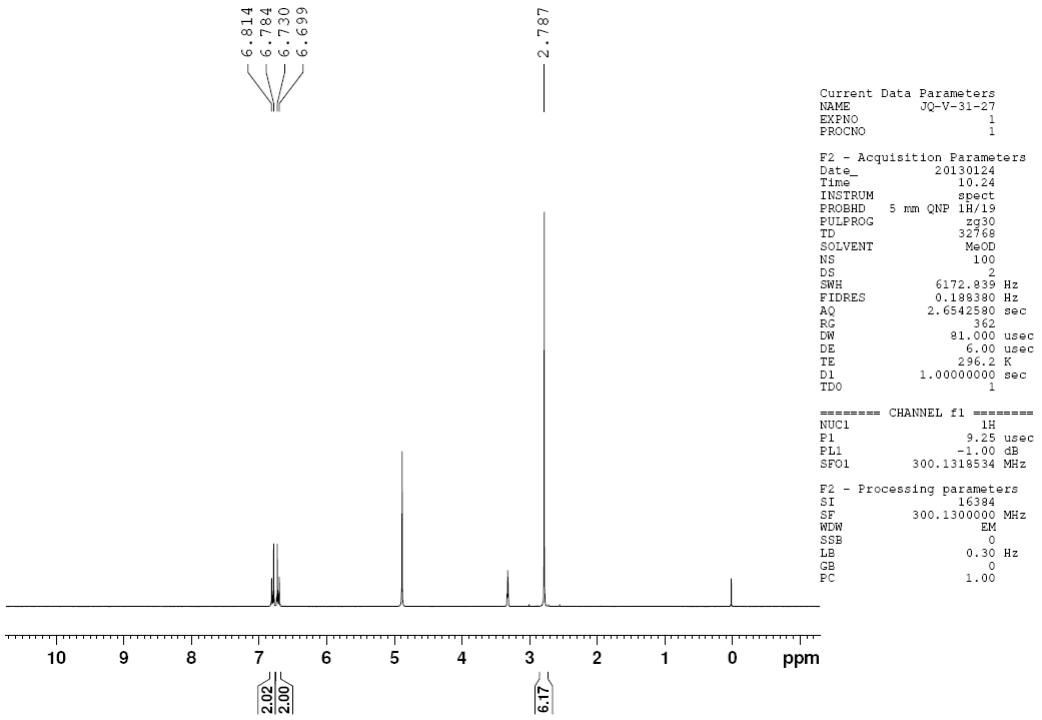
CypH-1

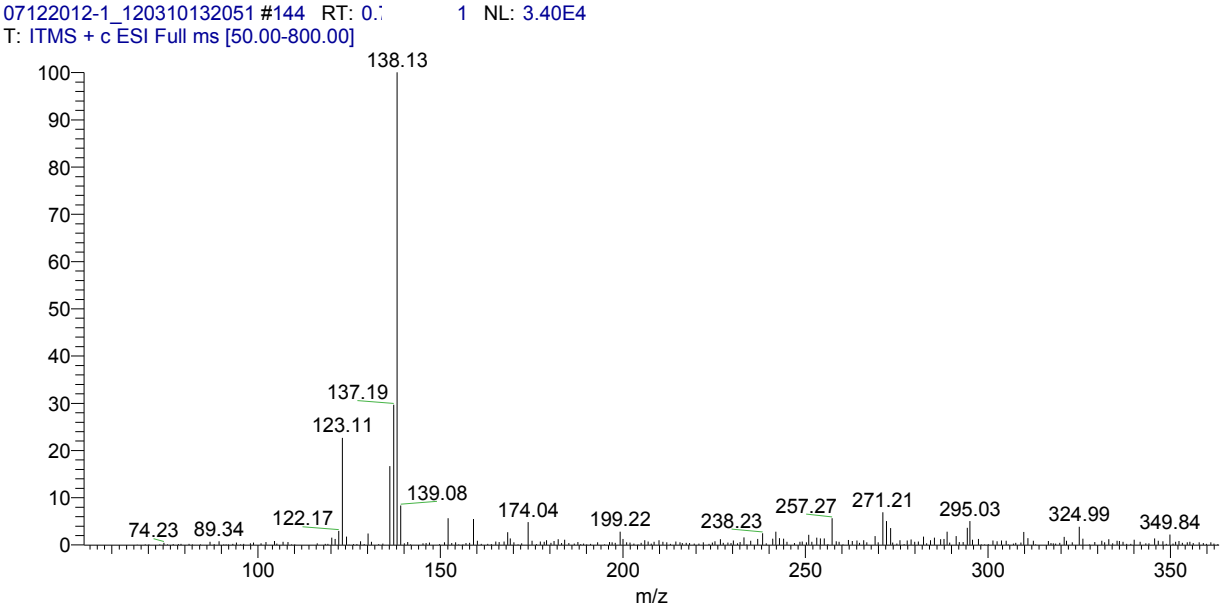


4-(Dimethylamino)phenol (57mg, 0.42mmol) in DMF (5 ml) was reacted with sodium hydride (17 mg) at RT for 15min. IR-775 (100 mg, 0.19 mmol, Aldrich, Dye content ~90%) was added slowly and stirred at RT overnight. The reaction was extracted with dichloromethane (DCM), and washed with brine. The product in DCM layer was purified by silica gel column using DCM/MeOH (MeOH 0-10%) as an elution solvent. The yield is 57 mg, 48 %. TLC: DCM/MeOH=10/1, R_f=0.25. ^{1}H-NMR (300 MHz, $CDCl_3$): 7.87 (2H, d, J=14.1 Hz), 7.71 (2H, d, J=7.8 Hz), 7.41-7.35 (2H, m), 7.31 (2H, d, J=6.3 Hz), 7.25 (2H, d, J=7.5Hz), 7.21-7.16 (2H, m), 7.09 (2H, d, J=7.8 Hz), 6.00 (2H, d, J=14.1 Hz), 3.58 (6H, s), 3.13 (6H, s), 2.75-2.65 (4H, m), 2.05-2.00 (2H,m), 1.36 (12H, s). ^{13}C-NMR (125MHz, MeOD): 172.77, 163.72, 158.83, 142.55, 141.99, 140.78, 139.21, 128.72, 125.44, 122.28, 122.08, 116.23, 110.26, 100.12, 49.02, 45.67, 31.22, 27.63, 24.18, 20.92. MS: 584 (M^{+}).

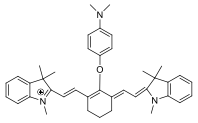
Reference: Bioconjugate Chem. 2011, 22, 2227-36.

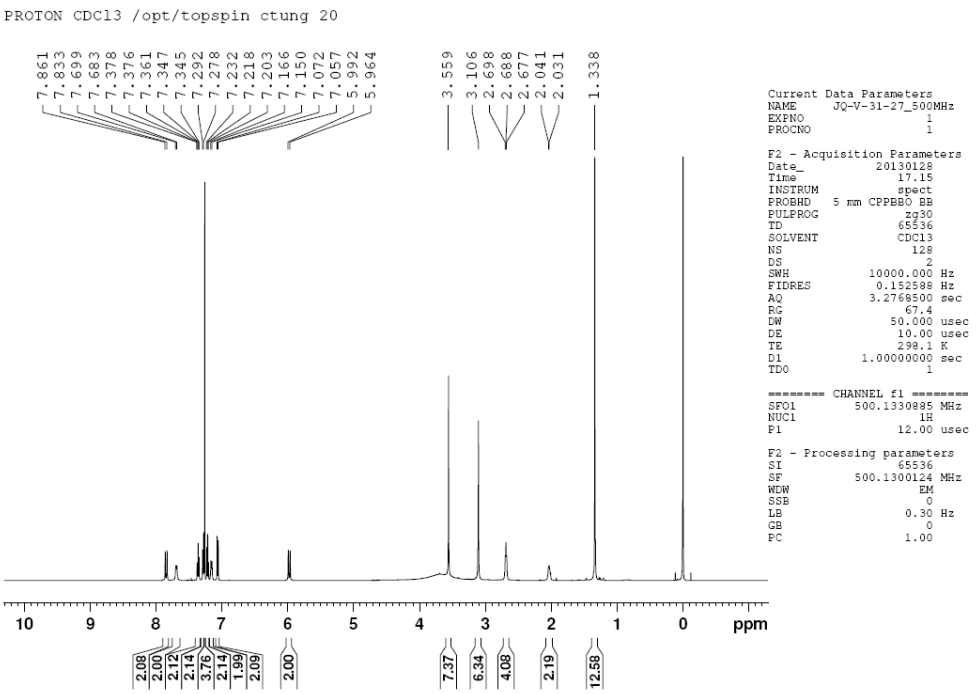
Spectra of 4-(dimethylamino)phenol

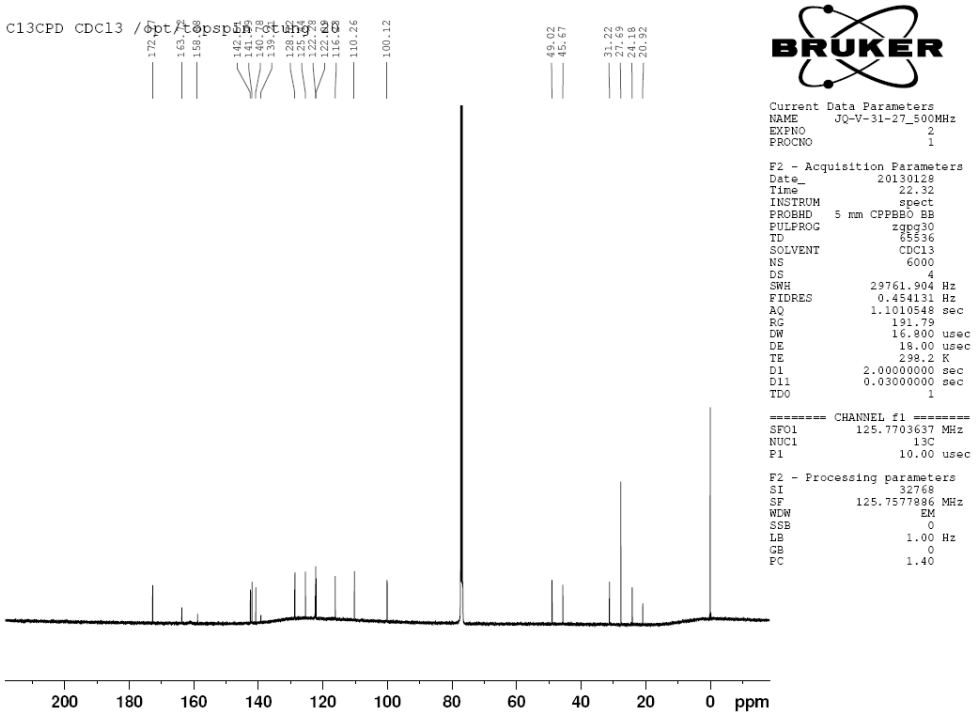


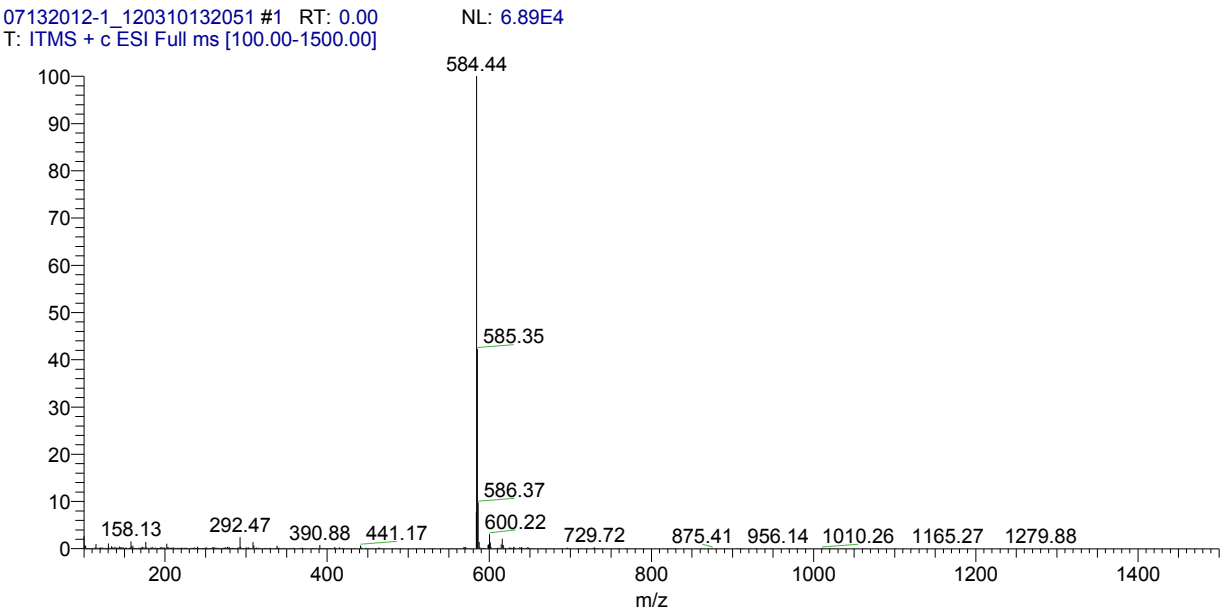


Spectra of CypH-1





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586.37
600.22
158.13
292.47
390.88
441.17
729.72
875.41
956.14
1010.26
1165.27
1279.88
100
90
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60
50
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30
20
10
0
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400
600
800
1000
1200
1400
m/z

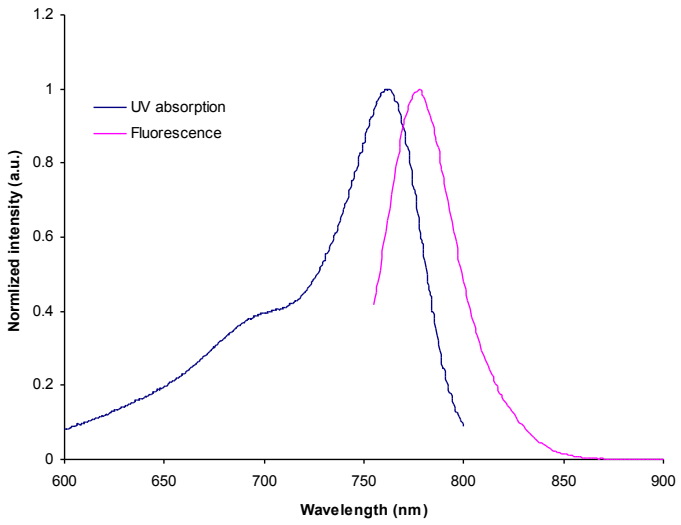

Fig S-1. Normalized absorption (blue) and emission (red) spectra of CypH-1 at pH=4 PBS buffer, Ex_{max} = 760 nm; Em_{max} = 777 nm.

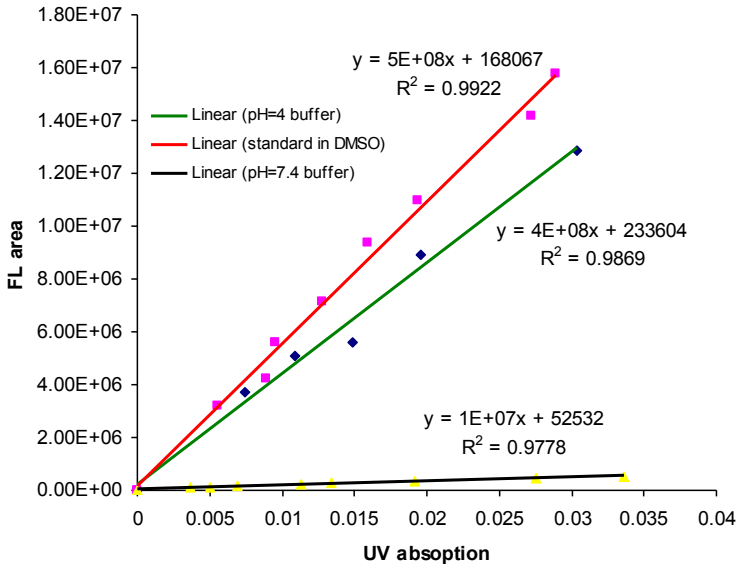

Fig S-2. Quantum yield measurement using indocyanine green in DMSO as a reference (Φ=0.106) following a published protocol (Pure Appl. Chem., 2011, 83, 12, 2213–2228).
$\Phi_{pH4.0} = \Phi_{Standard}[G_{radx}/G_{rads}][\eta_{H2O}/\eta_{DMSO}]^2 = 0.106[4\times108/5\times108][1.33/1.479]^2 = 0.06857$
$\Phi_{pH7.4} = \Phi_{Standard}[G_{radx}/G_{rads}][\eta_{H2O}/\eta_{DMSO}]^2 = 0.106[0.1\times108/5\times108][1.33/1.479]^2 = 0.001714$

LogP measurement

Standard curve preparation. CypH-1 stock solution (200μg/ml) was prepared in MeOH/H2O (1/3), then diluted to 100, 50, 10, 5 and 1μg/ml with pure water for a standard curve. The peak area at each concentration was determined using a reverse phase C18 column, (Phenomenex luna, 150X4.60 mm, 5μm) with mobile phase A: 0.1% TFA aqueous solution and mobile phase B: 0.1% TFA acetonitrile. The detector was set at 760 nm.

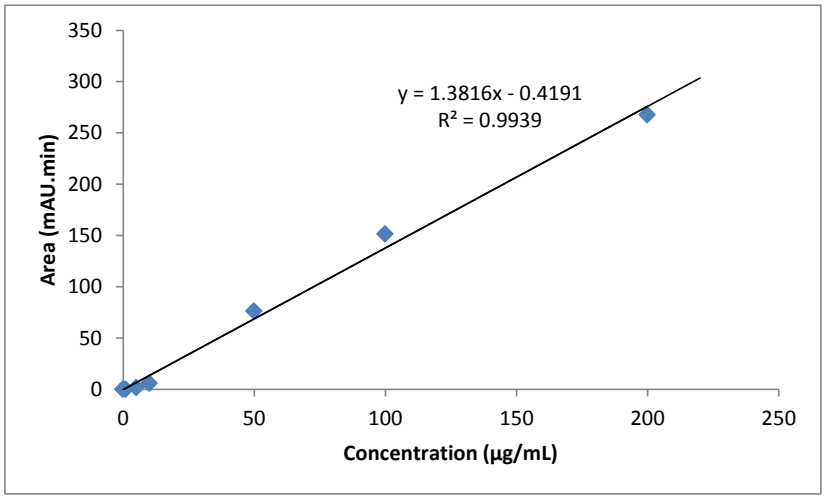

Fig S-3. The standard curve of CypH-1

		Peak area	Average Peak area (5 μL)	Concentration (μg/ml)
In octanol (injection 5 μL)	1	159.7	156.03	113.24
	2	154.3		
	3	154.1		
In pH=7.4 PBS buffer (injection 40 μL)	1	1.2	1.27/8 = 0.1588	0.42
	2	1.4		
	3	1.2		

Y=1.3816X-0.4191

$C_{(octanol)}$ = (156.03+0.4191)/1.3816 = 113.24 μg/ml

$C_{(water)}$ = (0.1588+0.4191)/1.3816 = 0.42μg/ml

P = $C_{(octanol)}/C_{(water)}$ = 113.24/0.42 = 269

logP = log[$C_{(octanol)}/C_{(water)}$] = 2.43