Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Supplement
to
pH-Sensitive fluorophores from locked GFP chromophores by a non-alternant analogue of the photochemical *meta* effect

By
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MP2/cc-pvdz structure (Å):

28			
MP2/CC-PVDZ ENERGY=-981.76666344			
B	-1.2828210418	0.1450915535	0.2411792667
C	-0.2693686379	0.0306859887	1.4935578017
C	1.1520849209	0.0704362399	1.3683719946
C	1.9818045787	0.0330903118	2.5186194226
C	1.4217173799	-0.0488115192	3.7935429121
C	0.0197219320	-0.1050311436	3.9310337458
C	-0.8025828865	-0.0676675671	2.7865484312
H	-1.8930174568	-0.1159996391	2.9105815160
O	-0.4774107288	-0.1948318662	5.1971143101
H	-1.4438872989	-0.2202375180	5.1259975872
H	2.0431743181	-0.0785975599	4.6934415159
H	3.0715656956	0.0634944547	2.4019426860
C	1.7576765184	0.0901635155	0.0566781917
C	1.0223121185	0.0211508994	-1.0966114110
N	-0.3797225868	-0.0577143212	-1.0983784564
C	-0.8036754384	-0.0946386881	-2.3531010882
N	0.2664391891	-0.0318923464	-3.2005861899
C	1.5076804386	-0.0064045394	-2.4885552259
O	2.6134437162	0.0140000702	-2.9976674304
O	0.1642566752	-0.2789173016	-4.5508242796
H	0.5296422729	0.5257641063	-4.9593767984
C	-2.2191581221	-0.1468030536	-2.8012264206
H	-2.5923019177	0.8811808390	-2.9459590007
H	-2.2931343912	-0.6901393141	-3.7536723637
H	-2.8187621612	-0.6290425267	-2.0185574872
H	2.8490842653	0.1342998789	-0.0503622942
F	-1.8668511482	1.4185144517	0.1056299405
F	-2.2728009015	-0.8571770850	0.2322244934

SA2-CAS(4,3)/cc-pvdz State Energies @ above structure:

!MCSCF STATE 1.1 Energy	-979.021497453775
!MCSCF STATE 2.1 Energy	-978.855937958705

SA2-CAS(4,3)/cc-pvdz state-averaged natural orbitals and occupation numbers:

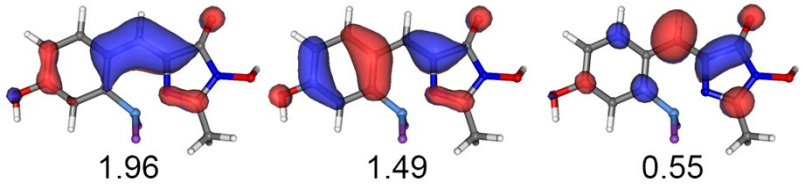

Table S2. Cartesian Coordinates (Å) of MP2/cc-pvdz-optimized ground state structure of *p*H-BDI-oBF_2-NOH-BDI (**VI**), SA2-CAS(4,3)/cc-pvdz state energies, natural orbitals and occupation numbers at MP2/cc-pvdz structure. All energies in atomic units (hartree).

MP2/cc-pvdz structure (Å):

```
   27
MP2/CC-PVDZ  ENERGY=-906.71474686
 B         -1.3551224641        0.1063183456        0.5025427701
 C         -0.4063670570       -0.0020758939        1.8040583473
 C          1.0194265180        0.0388217401        1.7545644556
 C          1.8000532690       -0.0017925332        2.9371434849
 H          2.8936789245        0.0304366059        2.8639659254
 C          1.1741121867       -0.0891421446        4.1860652183
 C         -0.2307502138       -0.1459010380        4.2557924664
 C         -0.9991807821       -0.1047821695        3.0773233703
 H         -2.0917483904       -0.1534255200        3.1486545898
 H         -0.7257296288       -0.2219624094        5.2303771337
 H          1.7747018707       -0.1193277431        5.1011601411
 C          1.6965533840        0.0736847282        0.4708805179
 C          1.0230539273        0.0162674036       -0.7172078885
 N         -0.3766595213       -0.0636977833       -0.7942497950
 C         -0.7329811759       -0.0801419562       -2.0688334276
 N          0.3791136812        0.0019164207       -2.8615537352
 C          1.5809160489        0.0110991571       -2.0857096183
 O          2.7125019072        0.0361140750       -2.5309254216
 O          0.3478469461       -0.2315554723       -4.2175725845
 H          0.6900292793        0.5946845839       -4.6023426914
 C         -2.1238361646       -0.1282327517       -2.5880263899
 H         -2.5040426405        0.9015562961       -2.6967824027
 H         -2.1440383824       -0.6244295121       -3.5681591571
 H         -2.7530713004       -0.6543876762       -1.8584647766
 H          2.7918656515        0.1225042082        0.4222556604
 F         -2.3179535308       -0.9171436990        0.4213466346
 F         -1.9556023194        1.3684695772        0.3418675063
```

SA2-CAS(4,3)/cc-pvdz state energies @ above structure:

```
!MCSCF STATE 1.1 Energy             -904.152278845704
!MCSCF STATE 2.1 Energy             -903.979074392594
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SA2-CAS(4,3)/cc-pvdz natural orbitals and occupation numbers:

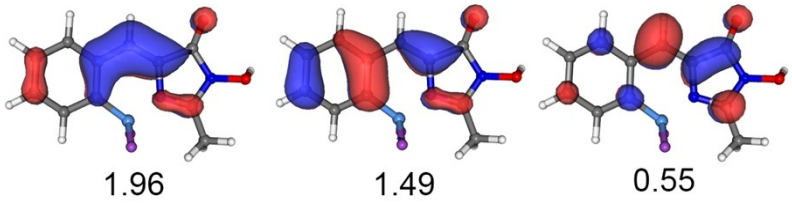

Table S3. Cartesian Coordinates (Å) of MP2/cc-pvdz-optimized ground state structure of *p*O^--BDI-oBF_2-NOH-BDI (**IX**), SA2-CAS(4,3)/cc-pvdz state energies, natural orbitals and occupation numbers at MP2/cc-pvdz structure. All energies in atomic units (hartree).

MP2/cc-pvdz ground state structure (Å):

```
   27
 MP2/CC-PVDZ  ENERGY=-981.23472746
 B         -1.2887613285        0.1648270579        0.2626397101
 C         -0.3048509807        0.0274210866        1.5409679915
 C          1.1463627031        0.0960656044        1.4080500134
 C          1.9763178052        0.0718515104        2.5904154613
 C          1.4281010460       -0.0432991641        3.8431307562
 C         -0.0272585067       -0.1520545753        4.0421499200
 C         -0.8300722550       -0.1055392457        2.8088504322
 H         -1.9166576684       -0.1753533611        2.9497756172
 O         -0.5338495038       -0.2704716112        5.1765346901
 H          2.0529596509       -0.0685209385        4.7443913912
 H          3.0665086804        0.1357962687        2.4612251143
 C          1.7350716855        0.1140963869        0.1449158110
 C          1.0000155165        0.0515337991       -1.0606944010
 N         -0.3931955071       -0.0330507832       -1.0627025667
 C         -0.8399299171       -0.1182252350       -2.3275617939
 N          0.2525916052       -0.0784918601       -3.1284184389
 C          1.4692692619        0.0047452235       -2.4182078162
 O          2.5514112828       -0.0012018118       -3.0530926064
 O          0.3113957705       -0.2037358778       -4.4983740548
 H          1.3020036978       -0.1900453326       -4.5665317146
 C         -2.2535433876       -0.1566805007       -2.7893670145
 H         -2.6332639119        0.8621593019       -2.9813954217
 H         -2.3289169251       -0.7394053654       -3.7213294554
 H         -2.8670644671       -0.6134889566       -2.0011203172
 H          2.8288672900        0.1551151314        0.0471219183
 F         -1.8785399034        1.4504514174        0.1458001694
 F         -2.3109001647       -0.8222079138        0.2315410490
```

SA2-CAS(4,3)/cc-pvdz state energies (a.u.) @ above structure:

```
!MCSCF STATE 1.1 Energy             -978.485966095080
!MCSCF STATE 2.1 Energy             -978.331240446186
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SA2-CAS(4,3)/cc-pvdz natural orbitals and occupation numbers:

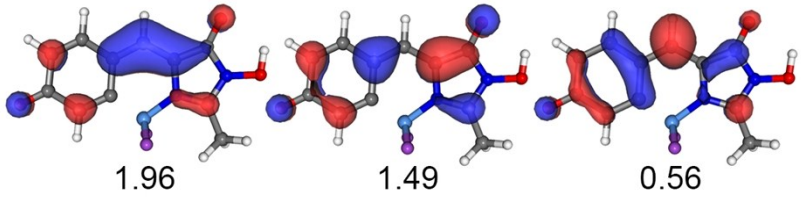

Table S4. Cartesian Coordinates (Å) of MP2/cc-pvdz-optimized ground state structure of *p*OH-BDI-oBF_2-NO^--BDI (**VIII**), SA2-CAS(4,3)/cc-pvdz state energies, natural orbitals and occupation numbers at MP2/cc-pvdz structure; SA3-CAS(6,4)/cc-pvdz natural orbitals and occupation numbers. All energies in atomic units (hartree).

MP2/cc-pvdz ground state structure (Å):

```
   27
 MP2/CC-PVDZ  ENERGY=-981.23018773
 B         -1.2588388060        0.0000123507       -0.1708443727
 C         -0.2465488269       -0.0000003392       -1.4453225744
 C          1.1771320756        0.0000013187       -1.3315802790
 C          1.9881804768        0.0000039525       -2.4946410175
 C          1.4164827726        0.0000031270       -3.7716535019
 C          0.0149204336       -0.0000016419       -3.8960417277
 C         -0.7893129686       -0.0000073487       -2.7435520025
 H         -1.8834760029       -0.0000147957       -2.8569454194
 O         -0.5136188383       -0.0000055079       -5.1706567309
 H         -1.4751656221       -0.0000008408       -5.0545719682
 H          2.0319365393        0.0000096085       -4.6775253996
 H          3.0806315745        0.0000105752       -2.3887205775
 C          1.7865198626        0.0000006937       -0.0108477582
 C          1.0107772636       -0.0000233950        1.1173146444
 N         -0.3698108839       -0.0000485514        1.1128147456
 C         -0.7714010124       -0.0000232477        2.4301269096
 N          0.2554999752        0.0000219254        3.3051835416
 C          1.4867273510       -0.0000092182        2.5250407871
 O          2.6147577463       -0.0000122818        2.9910865469
 O          0.2151799430        0.0000233270        4.5869928101
 C         -2.1843019456       -0.0000367544        2.8818783564
 H         -2.7068518849       -0.8870363663        2.4884862068
 H         -2.1755553422       -0.0005674396        3.9818255606
 H         -2.7065977423        0.8875425842        2.4894390703
 H          2.8757420683        0.0000177051        0.1146946427
 F         -2.1007001769       -1.1540981849       -0.1662613976
 F         -2.1006217007        1.1541733426       -0.1662008568
```

SA2-CAS(4,3)/cc-pvdz state energies @ above structure:

```
!MCSCF STATE 1.1 Energy             -978.457618378426
!MCSCF STATE 2.1 Energy             -978.355140116831
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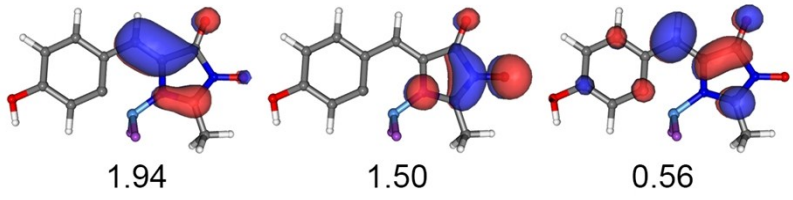
SA2-CAS(4,3)/cc-pvdz natural orbitals and occupation numbers:



SA3-CAS(6,4)/cc-pvdz state energies @ above structure:

```
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!MCSCF STATE 2.1 Energy             -978.340379936465
!MCSCF STATE 3.1 Energy             -978.276515722728
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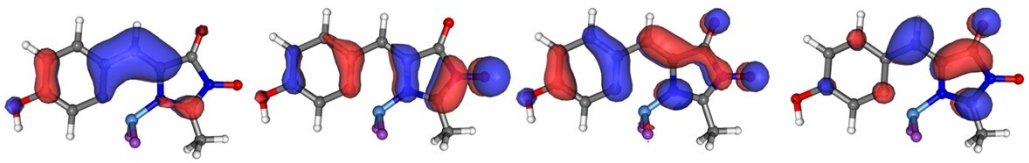
SA3-CAS(6,4)/cc-pvdz natural orbitals & occupation numbers:



1.98 1.68 1.61 0.72

Table S5. Cartesian Coordinates (Å) of MP2/cc-pvdz-optimized ground state structure of *p*H-BDI-oBF_2-NO^--BDI (*NO*$^-$ base conjugate to **VI**, not explicitly named in the text), SA2-CAS(4,3)/cc-pvdz state energies, natural orbitals and occupation numbers at MP2/cc-pvdz structure. SA3-CAS(6,4)/cc-pvdz natural orbitals and occupation numbers. All energies in atomic units (hartree).

MP2/cc-pvdz ground state structure (Å):

```
   26
 MP2/CC-PVDZ ENERGY=-906.17982590
 B          -1.3292129570        0.0001502548       -0.4242195494
 C          -0.3929321932        0.0000004214       -1.7559939079
 C           1.0357519565        0.0000492978       -1.7233582366
 C           1.7905240672       -0.0000042838       -2.9243337707
 H           2.8868639677        0.0000373286       -2.8676975154
 C           1.1479951904       -0.0001200467       -4.1686426405
 C          -0.2599027572       -0.0002012129       -4.2222178935
 C          -1.0035478657       -0.0001450604       -3.0284761963
 H          -2.0993416485       -0.0002155049       -3.0805826339
 H          -0.7713232025       -0.0003087224       -5.1927513066
 H           1.7379923440       -0.0001543048       -5.0930714270
 C           1.7200646766        0.0001041013       -0.4377198943
 C           1.0100016273        0.0000061069        0.7325326347
 N          -0.3667960828       -0.0001232616        0.8088223013
 C          -0.6912682865       -0.0001452210        2.1475412472
 N           0.3838050511       -0.0000632660        2.9622206444
 C           1.5671973306        0.0000061698        2.1120721670
 O           2.7203781924        0.0000576941        2.5107257960
 O           0.4182845835       -0.0001077091        4.2442035961
 C          -2.0760124095       -0.0002187604        2.6788846647
 H          -2.6194789051       -0.8870578959        2.3146855239
 H          -2.0056586998       -0.0007001608        3.7765484039
 H          -2.6192580056        0.8871166039        2.3155435640
 H           2.8146781289        0.0001935323       -0.3745793661
 F          -2.1660719840        1.1552449802       -0.3652471208
 F          -2.1665949270       -1.1545722479       -0.3653598010
```

SA2-CAS(4,3)/cc-pvdz state energies @ above structure:

```
!MCSCF STATE 1.1 Energy             -903.589936277323
!MCSCF STATE 2.1 Energy             -903.490042645950
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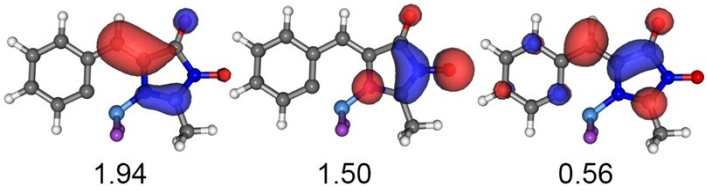
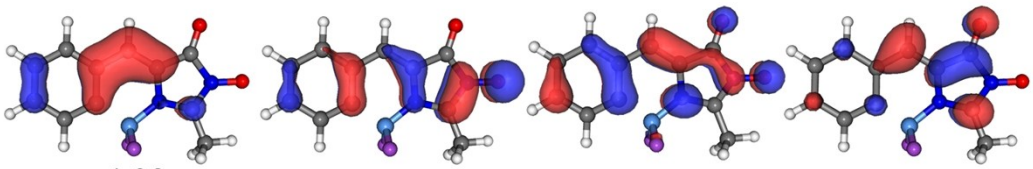
SA2-CAS(4,3)/cc-pvdz natural orbitals & occupation numbers:



SA3-CAS(6,4)/cc-pvdz state energies @ above structure:

```
!MCSCF STATE 1.1 Energy             -903.596407595891
!MCSCF STATE 2.1 Energy             -903.475424305193
!MCSCF STATE 3.1 Energy             -903.406293051620
```

SA3-CAS(6,4)/cc-pvdz natural orbitals & occupation numbers:


1.98
1.68
1.61
0.72

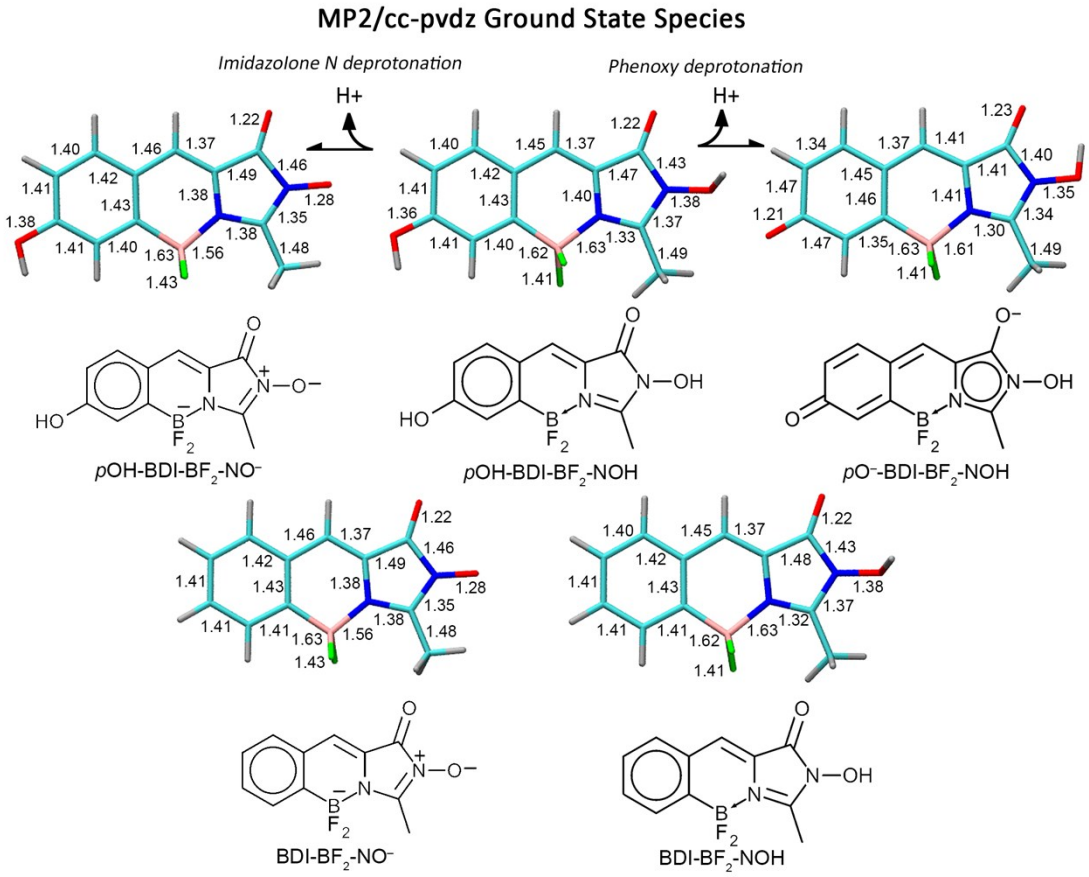


Figure S1. Geometries of species for which theoretical data is reported in Table 3 of the main text. Geometries were determined by optimization on the ground state of an MP2/cc-pvdz model (see text). Heavy (i.e. not H) bond lengths are shown in Å. The figure shows why we chose to denote the NB bond as coordinate (dative) in the text. Deprotonation of the NOH leads to a ~0.1 Å contraction of the NB bond. Since NOH is also expected to stabilize the covalent bonding form (see figure), we deduced that the protonated species was best drawn as having a dative NB bond.

II. Spectroscopic and other data

II.1 Optical properties

Solvent	V		IV		VI	
	Abs max	Ext coef	Abs max	Ext coef	Abs max	Ext coef
H_2O/MeOH	371	18000	416	22500	409	19000
H_2O	369	18000	411	22000	405	n.d.[a]
EtOH	368	20500	421	23500	407	22000
CH_3CN	365	22500	407	24000	402	22000

a – is not soluble enought

II.2 Titration curves (absorbtion)

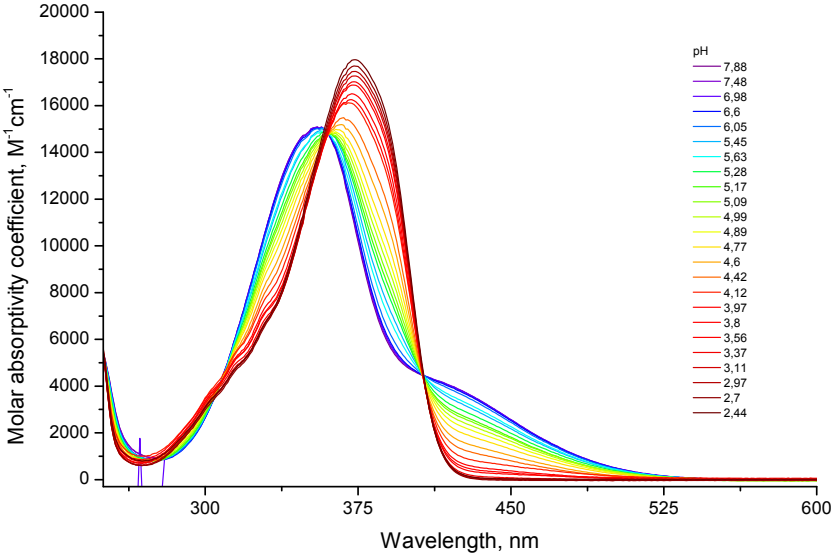


Figure 1. Absorption spectra of compound **V** at different pH in phosphate buffer (50 mM).

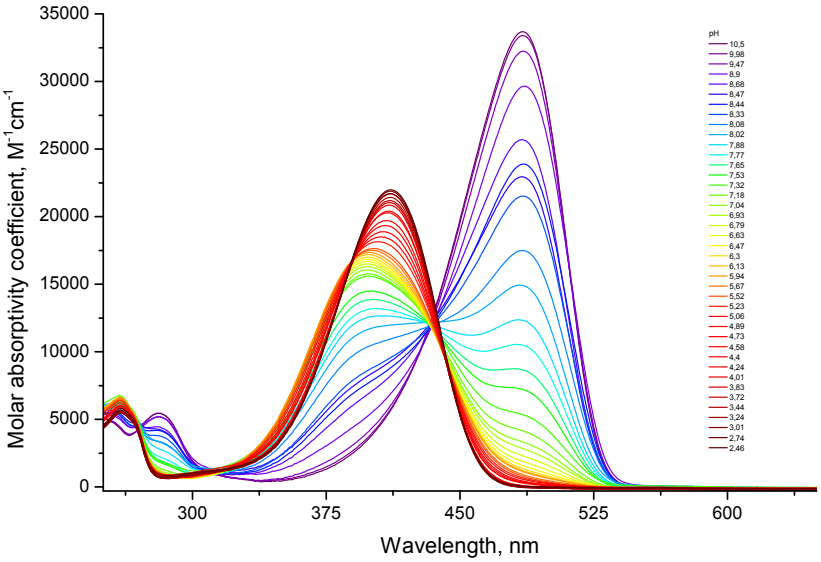


Figure 2. Absorption spectra of compound **IV** at different pH in phosphate buffer (50 mM).

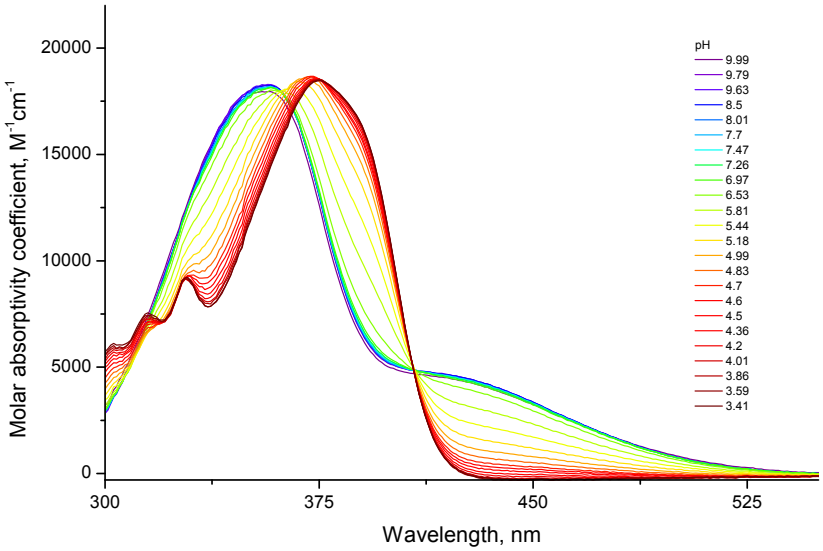


Figure 5. Absorption spectra of compound **V** at different pH in water-methanol mixture.

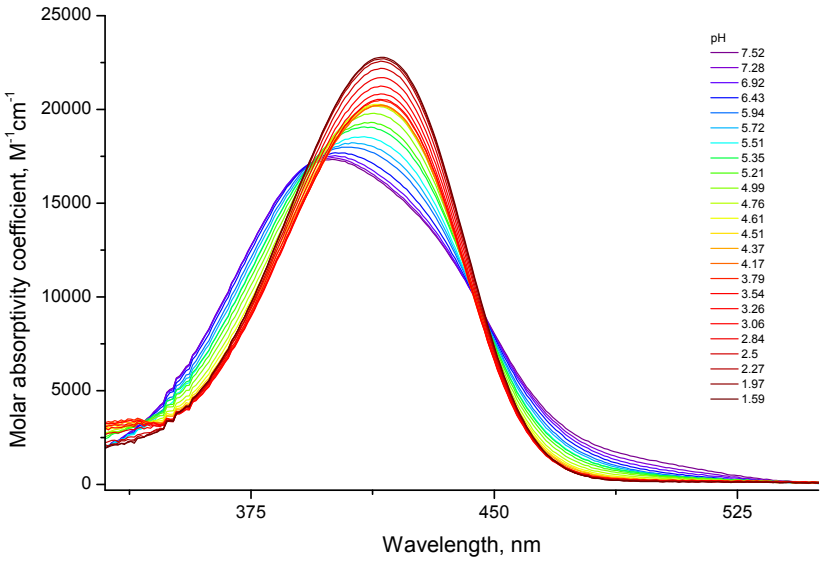


Figure 6. Absorption spectra of compound **IV** at different pH in water-methanol mixture.

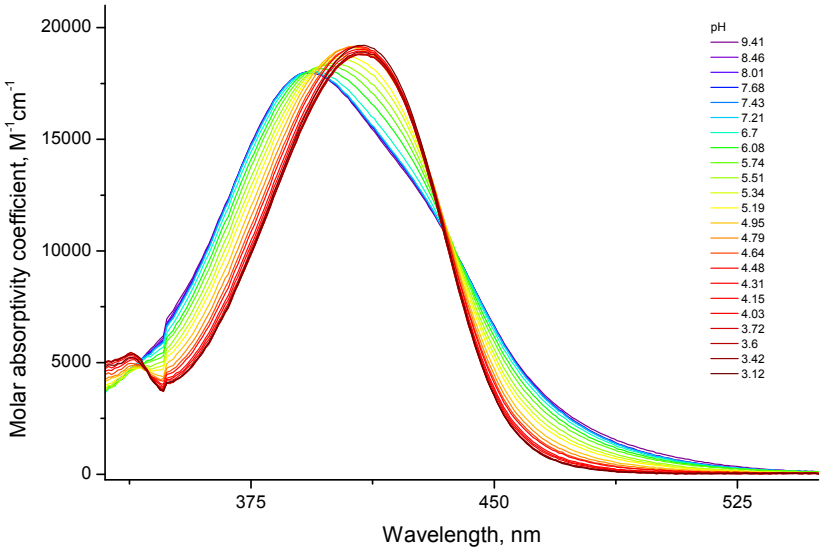


Figure 7. Absorption spectra of compound **VI** at different pH in water-methanol mixture.

II.3 Titration curves (emission)

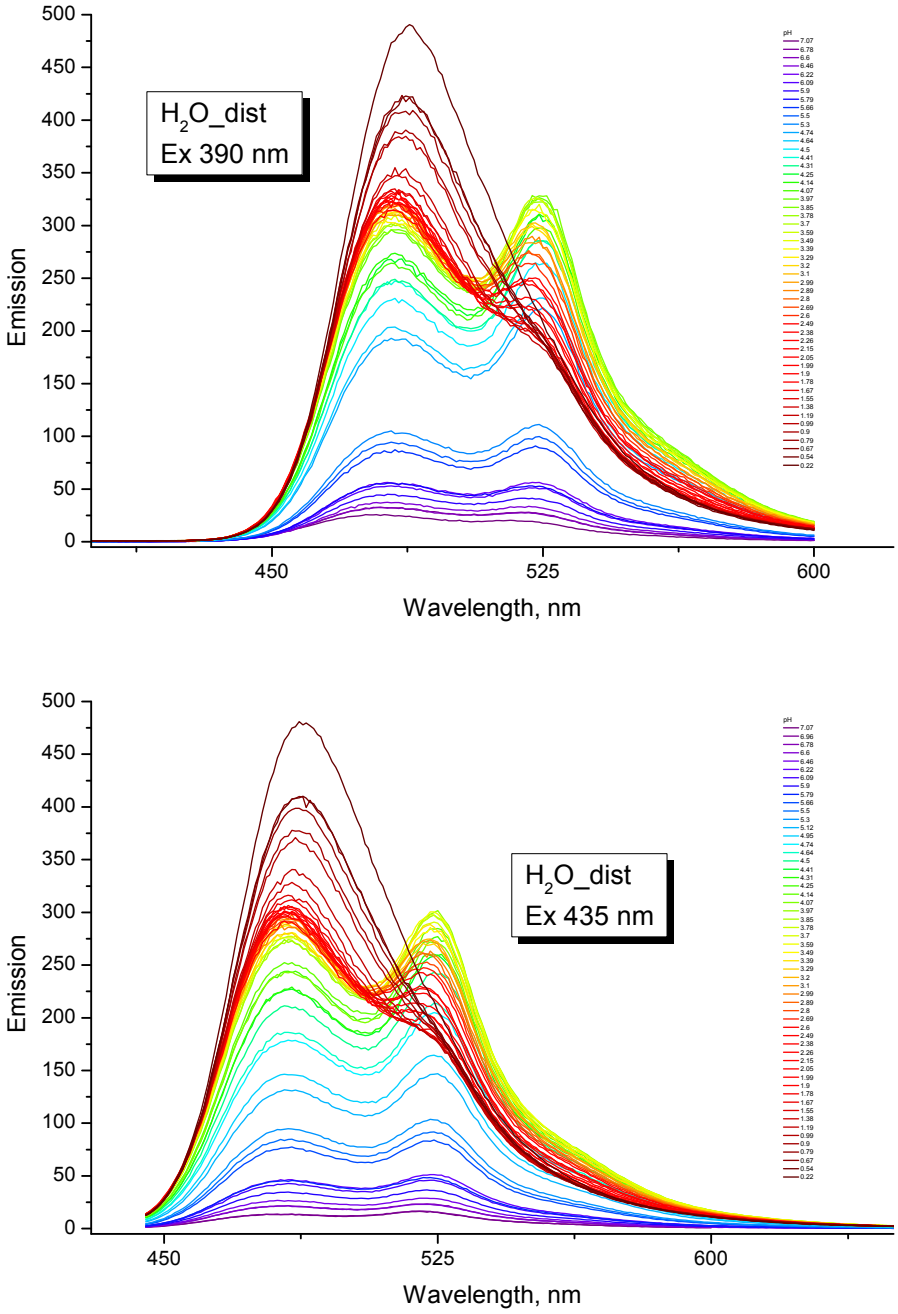


Figure 9. Emission spectra of compound **IV** at different pH in water.

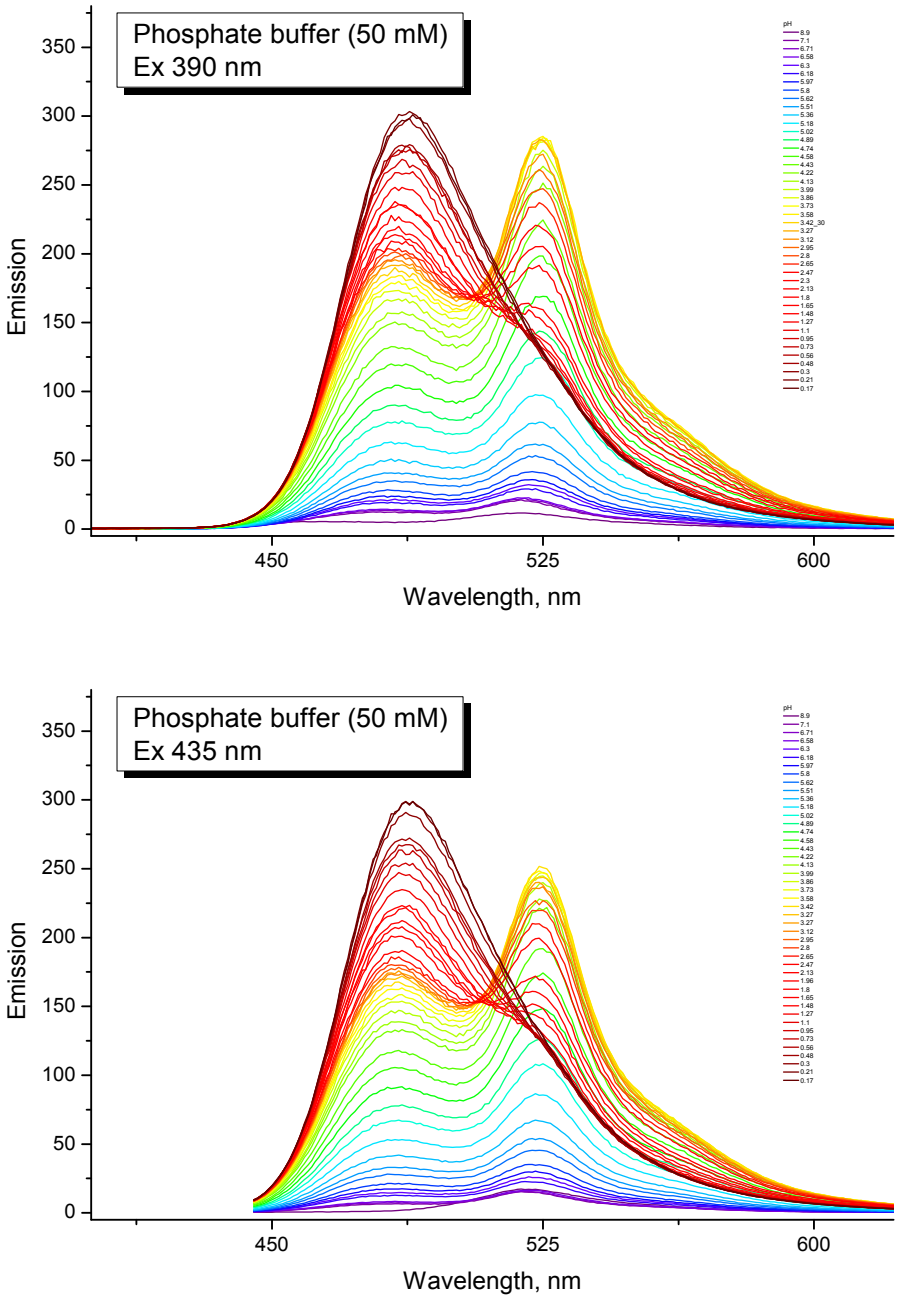
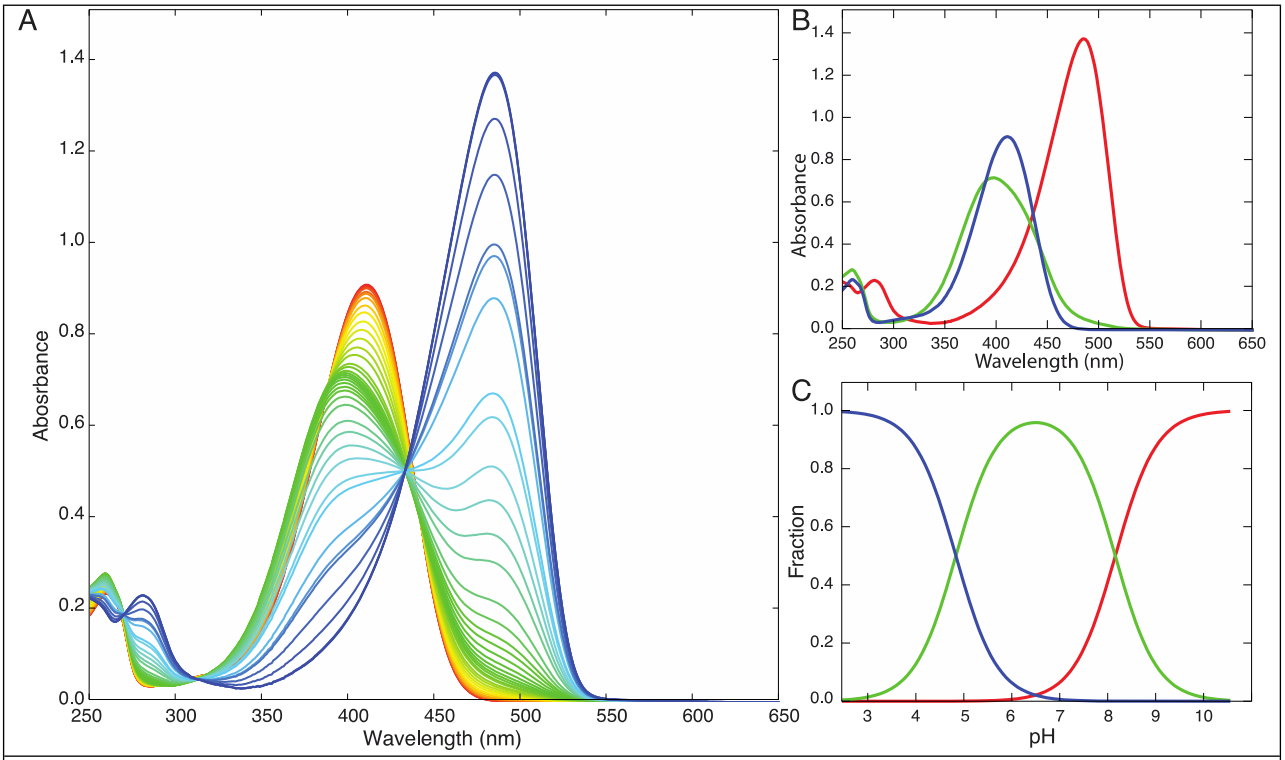


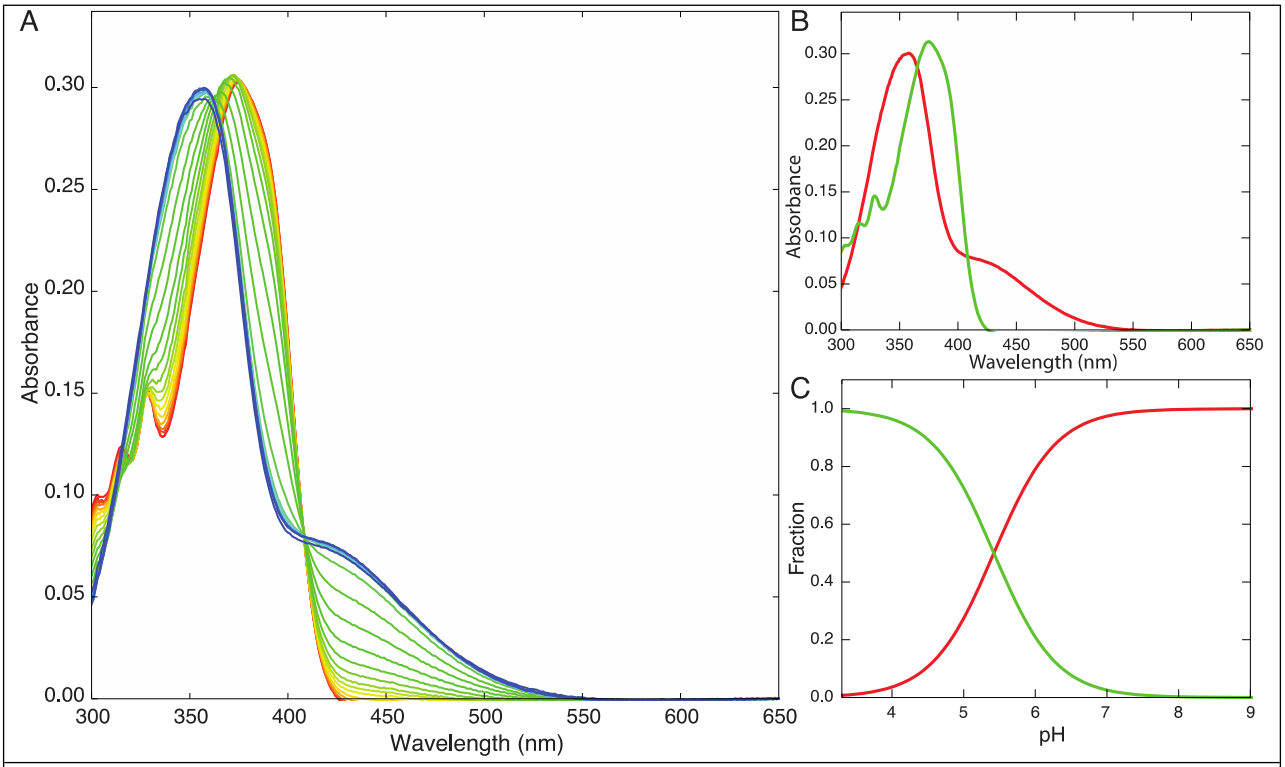
Figure 10. Emission spectra of compound **IV** at different pH in phosphate buffer.

III. Summary of SVD Analysis

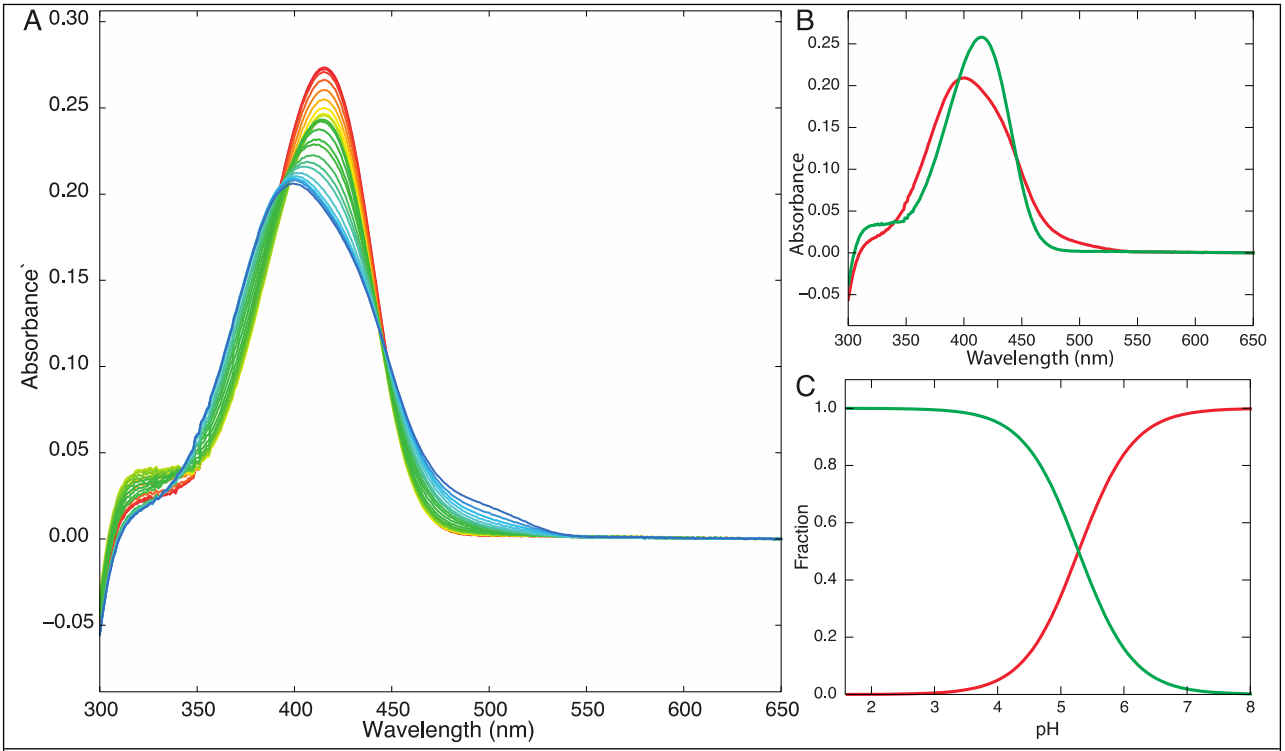
KinTek Explorer software (www.kintekcorp.com, KinTek Corp, Austin, TX) was used to de-convolute changes in spectra as a function of pH based upon singular value decomposition analysis in order to derived the pH dependence and spectra of individual species. Fitting by nonlinear regression included weighting to give preference to the most significant Eigenvectors. Fitting to each model was based upon numerical integration of the rate equations to derive the endpoint of the reaction at each pH. The figures below show the original pH dependence of the emission spectra and the derived emission spectra and pH dependence of each species.



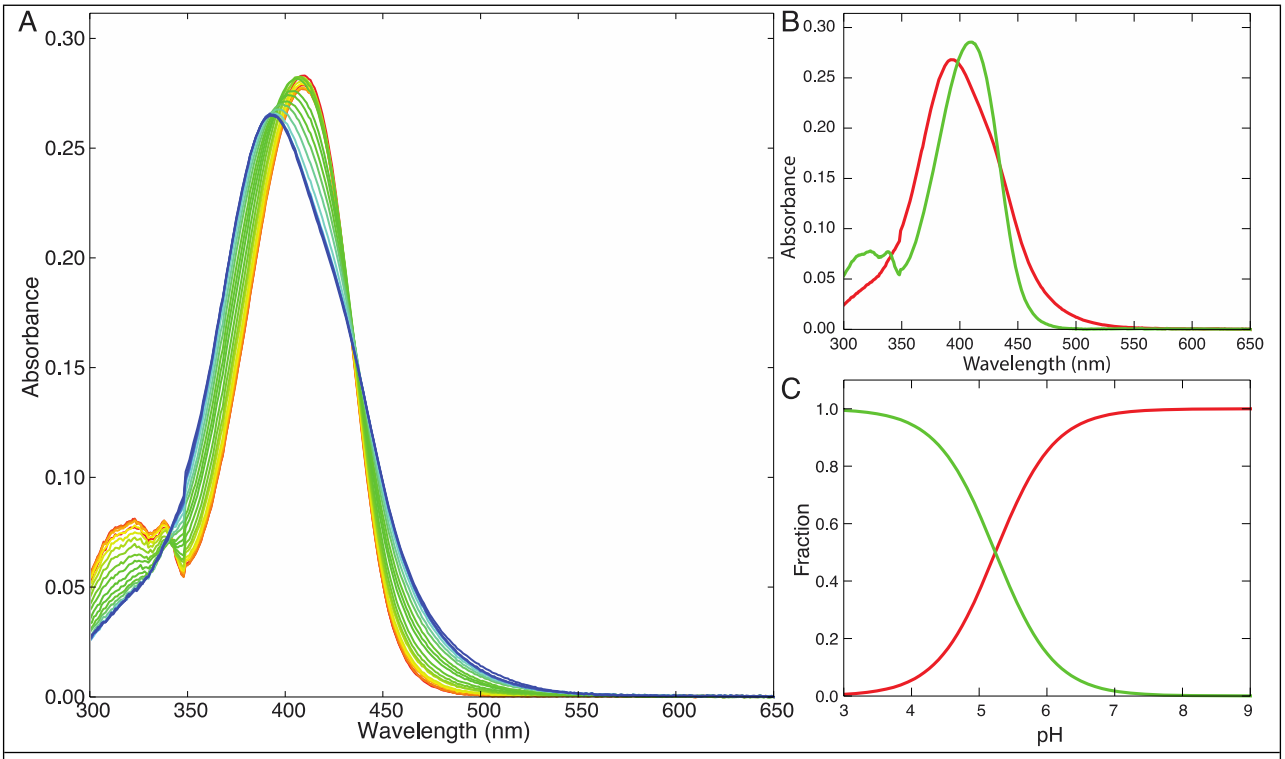
Absorption spectra of compound **IV** at different pH in phosphate buffer
A. pH dependence of the observed absorption spectra; the red to blue color scale corresponds to pH 2.5 to 10.5. **B**. Spectra of each species. **C**. pH dependence of each species defining pK_a values of 4.83 ± 0.03 and 8.16 ± 0.02.



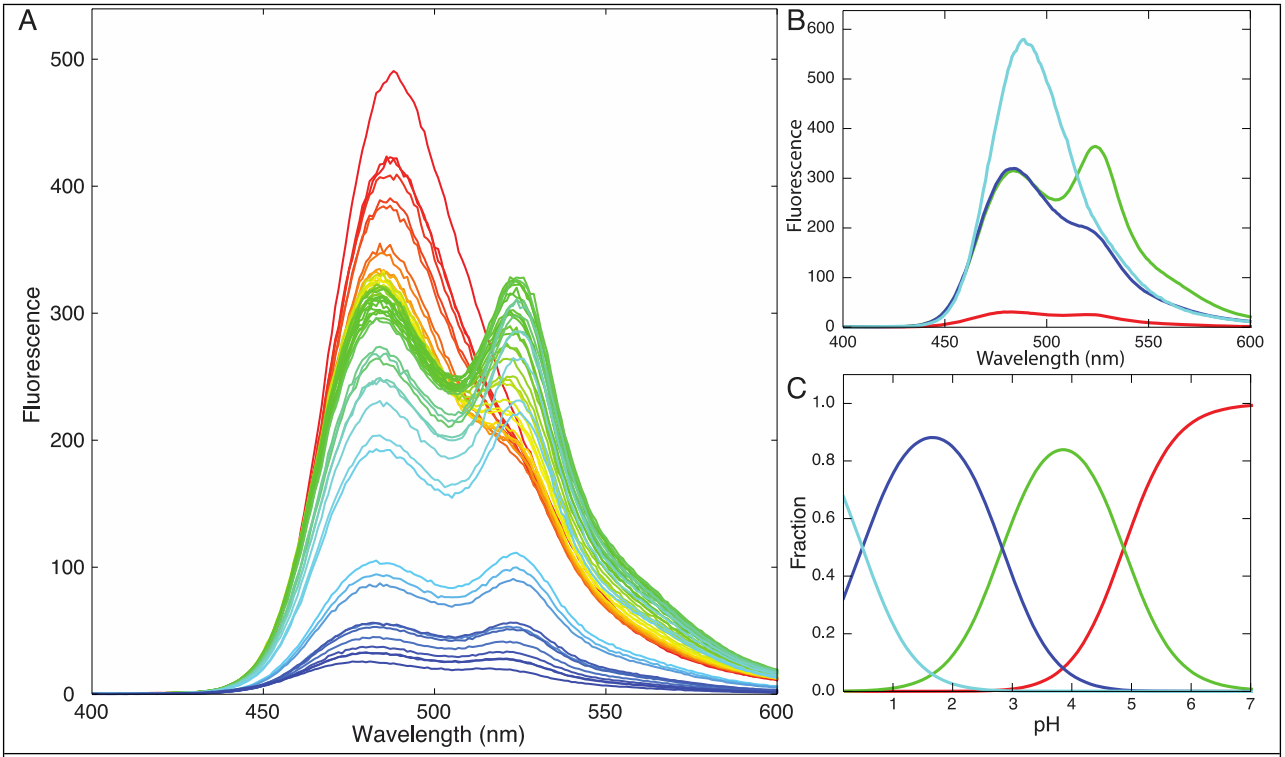
Absorption spectra of compound **V** at different pH in water-methanol mixture
A. pH dependence of the observed absorption spectra; the red to blue color scale corresponds to pH 3.4 to 9. **B**. Spectra of each species. **C**. pH dependence defining a pK_a value of 5.42 ± 0.02.



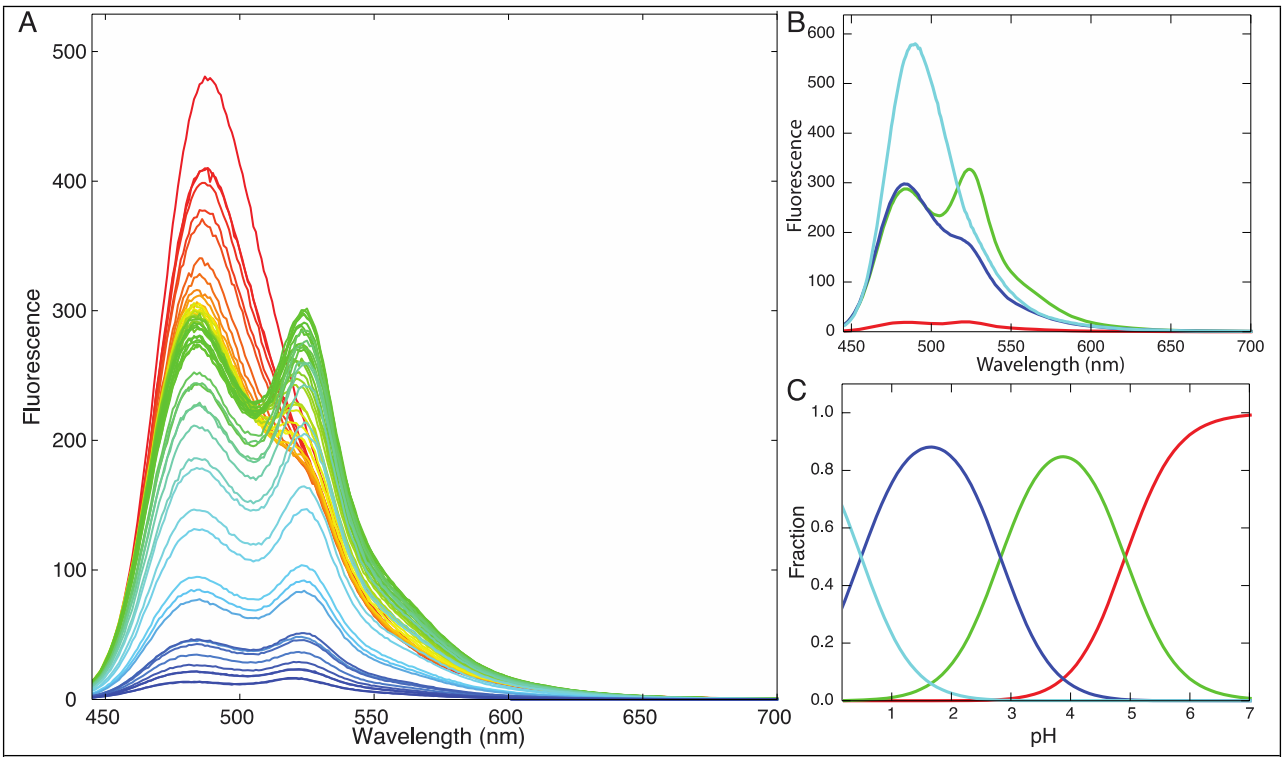
Absorption spectra of compound **IV** at different pH in water-methanol mixture
A. pH dependence of the observed absorption spectra; the red to blue color scale corresponds to pH 1.6 to 7.8. **B**. Spectra of each species. **C**. pH dependence of each species defining a pK_a value of 5.28 ± 0.09. Data above pH 8 suggesting a possible second deprotonation event were omitted from the analysis.



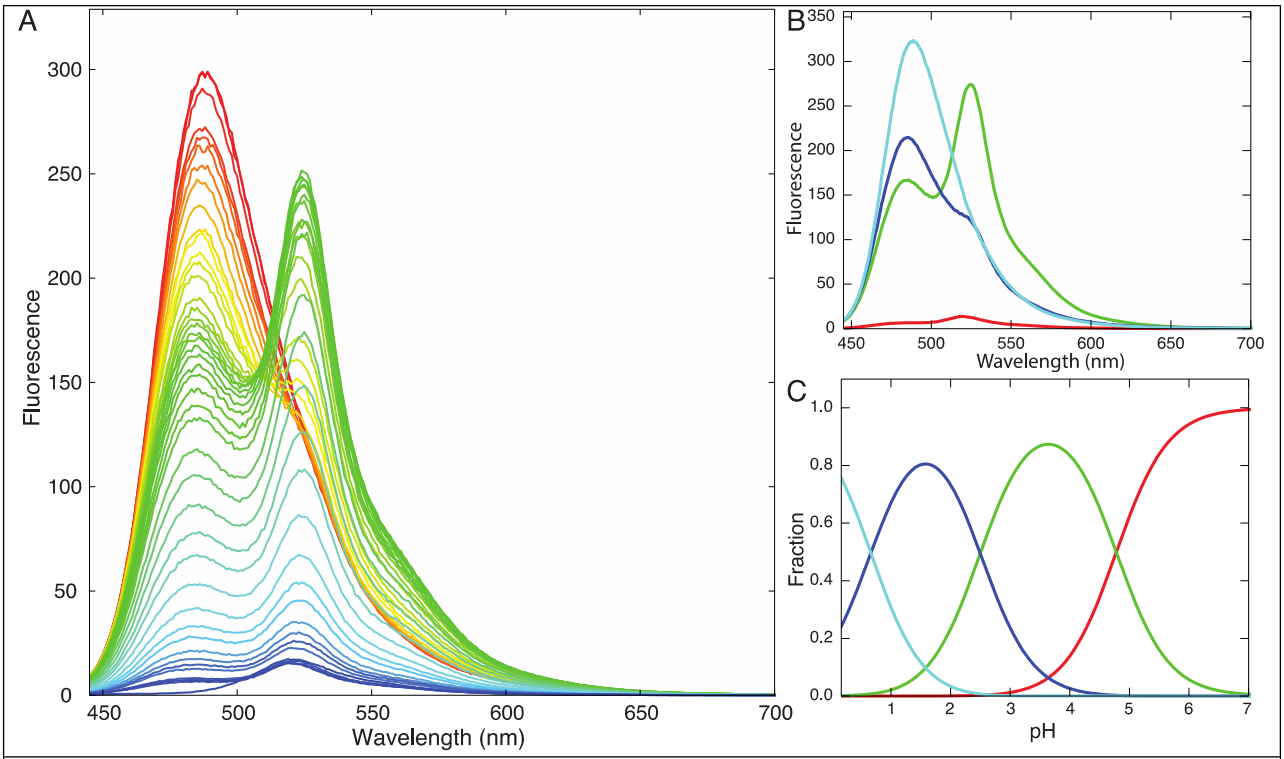
Absorption spectra of compound **VI** at different pH in water-methanol mixture
A. pH dependence of the observed absorption spectra; the red to blue color scale corresponds to pH 3.1 to 9. **B**. Spectra of each species. **C**. pH dependence defining a pK_a value of 5.24 ± 0.02.



Emission spectra of compound **IV** at different pH in water (excitation on 390 nm).
A. pH dependence of the observed emission spectra; the red to blue color scale corresponds to pH 0.2 to 7.0. **B**. Spectra of each species. **C**. pH dependence of each species defining pK_a values of 0.49 ± 0.13, 2.84 ± 0.08 and 4.87 ± 0.03.



Emission spectra of compound **IV** at different pH in water (excitation on 435 nm).
A. pH dependence of the observed emission spectra; the red to blue color scale corresponds to pH 0.2 to 7. **B**. Spectra of each species. **C**. pH dependence of each species defining pK_a values of 0.49 ± 0.1, 2.83 ± 0.08 and 4.92 ± 0.03.



Emission spectra of compound **IV** at different pH in phosphate buffer (excitation on 435 nm).
A. pH dependence of the observed emission spectra; the red to blue color scale corresponds to pH 0.2 to 7. **B**. Spectra of each species. **C**. pH dependence of each species defining pK_a values of 0.66 ± 0.1, 2.50 ± 0.04 and 4.78 ± 0.01.

IV. Kamlet-Taft analysis of quantum yield data

In order to better understand the dependence of the quantum yield data for **IV** in Table 1, we performed a multivariate linear regression of logQY for this species against the Kamlet-Taft solvatochromic parameters for the solvents described. The Kamlet-Taft parameters α, β, and π* for the solvents were taken from reference 34. The resulting fit had correlation coefficient R=0.99 and was found to be $\log QY = 1.0 - 0.1\alpha - 1.6\beta - 1.1\pi^*$. The parameters α, β, and π* are measures of the relative hydrogen bond donation (acidity), hydrogen bond acceptance (basicity) and polar solvation power of the solvents used. For more detailed background see reference 34. We emphasize that there are only five data points for **IV** in Table 1 against which the fit was taken. It should be interpreted with extreme caution. With this in mind, the analysis does support the assertion that increases in both solvent basicity (β) and polarity (π*) both kill the QY more effectively than do increases in the solvent acidity (α).