Electronic Supplementary Information for Substituent Effects on the Turn-on Kinetics of Rhodamine-based Fluorescent pH Probes

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Derivation of Text Eq 2

 $CF + H^+ \xrightarrow{k_1} CFH^+ \xrightarrow{k_2} OFH^+$

$$\frac{d[OFH^+]}{dt} = k_2[CFH^+] - k_{-2}[OFH^+]$$
(S-1)

If we assume the first reaction step is in rapid equilibrium, then:

$$k_1[CF][H^+] = k_{-1}[CFH^+]$$
(S-2)

Substituting the expression for [CFH⁺] derived from S-2 into S-1 gives S-3:

$$\frac{d[OFH^+]}{dt} = k_2 \left[\frac{k_1[CF][H^+]}{k_{-1}} \right] - k_{-2}[OFH^+]$$
(S-3)

We can define total dye concentration, [D], as:

$$[D] = [CF] + [CFH^+] + [OFH^+]$$
(S-4a)

Substituting in the expression for [CFH⁺], rearranging, and solving for [CF] gives:

$$[CF] = \frac{k_{-1}([D] - [OFH^+])}{k_{-1} + k_1[H^+]}$$
(S-4b)

Substituting S-4b into S-3 gives:

$$\frac{d[OFH^+]}{dt} = \frac{k_2 k_1}{k_{-1}} [H^+] \left(\frac{k_{-1} ([D] - [OFH^+])}{k_{-1} + k_1 [H^+]} \right) - k_{-2} [OFH^+]$$
(S-5a)

Eq S-5b defines the constant Q:

$$Q = \frac{k_2 k_1 [H^+]}{k_{-1} + k_1 [H^+]}$$
(S-5b)

$$\frac{d[OFH^+]}{dt} = -(Q + k_{-2})[OFH^+] + Q[D]$$
(S-5c)

S-5c is a first order non-homogeneous differential equation for which the solution is given by S-6, which is the same as eq 2 in the main text:

$$[OFH^+] = \frac{Q[D]}{Q+k_{-2}} \left(1 - e^{-(Q+k_{-2})t}\right)$$
(S-6)

Derivation of text Eq 4

At equilibrium:

$$k_1[CF][H^+] = k_{-1}[CFH^+]$$
 (S-7a)

$$K_1 = \frac{k_1}{k_{-1}} = \frac{[CFH^+]}{[CF][H^+]}$$
 (S-7b)

Also at equilibrium:

$$k_2[CFH^+] = k_{-2}[OFH^+]$$
 (S-8a)

$$[CFH^+] = \frac{k_{-2}[OFH^+]}{k_2}$$
(S-8b)

Substituting S-8b into S-7b gives:

$$K_1 = \frac{k_{-2}[OFH^+]}{k_2[CF][H^+]}$$
(S-9a)

$$[H^+] = \frac{1}{K_1} \cdot \frac{k_{-2}[OFH^+]}{k_2[CF]}$$
(S-9b)

 $1/K_{1}$ is essentially the acid dissociation constant $K_{a},$ so:

$$[H^+] = K_a \cdot \frac{k_{-2}[OFH^+]}{k_2[CF]}$$
(S-9c)

$$pH = pK_a - \log\left(\frac{k_{-2}[OFH^+]}{k_2[CF]}\right)$$
 (S-9d)

$$pH = pK_a + \log\left(\frac{k_2[CF]}{k_{-2}[OFH^+]}\right)$$
(S-9e)

Eq S-9e is eq 4 from the main text.



Figure S-1. Absorbance and fluorescence ($\lambda_{exc} = 535$ nm) spectra of the indicated compounds at 7.7 μ M (1-5) or 0.077 μ M (6-8) in 1:1 (v/v) ethanol:water as the pH was lowered by addition of aliquots of HCl as described in the Experimental Section.



Figure S-2. Absorbance and fluorescence ($\lambda_{exc} = 535 \text{ nm}$) spectra of compound **9** at 0.077 μ M in 1:1 (v/v) ethanol:water as the pH was lowered by addition of aliquots of HCl as described in the Experimental Section.







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