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

\[
\text{CF} + \text{H}^+ \xrightleftharpoons[k_-]{k_1} \text{CFH}^+ \xrightleftharpoons[k_-]{k_2} \text{OFH}^+ 
\]

\[
\frac{d[\text{OFH}^+]}{dt} = k_2[\text{CFH}^+] - k_-\text{[OFH}^+] 
\]

(S-1)

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

\[
k_1[CF][H^+] = k_-\text{[CFH}^+] 
\]

(S-2)

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

\[
\frac{d[\text{OFH}^+]}{dt} = k_2 \left[\frac{k_1[CF][H^+]}{k_-}\right] - k_-\text{[OFH}^+] 
\]

(S-3)
We can define total dye concentration, [D], as:

\[ [D] = [CF] + [CFH^+] + [OFH^+] \]  \hspace{1cm} (S-4a)

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

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

Substituting S-4b into S-3 gives:

\[ \frac{d[OFH^+]}{dt} = \frac{k_2k_1}{k_{-1}k_1}[H^+] \left( \frac{k_{-1}([D]-[OFH^+])}{k_{-1} + k_1[H^+]} \right) - k_{-2}[OFH^+] \]  \hspace{1cm} (S-5a)

Eq S-5b defines the constant Q:

\[ Q = \frac{k_2k_1[H^+]}{k_{-1} + k_1[H^+]} \]  \hspace{1cm} (S-5b)

\[ \frac{d[OFH^+]}{dt} = -(Q + k_{-2})[OFH^+] + Q[D] \]  \hspace{1cm} (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:

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

**Derivation of text Eq 4**

At equilibrium:

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

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

Also at equilibrium:

\[ k_2[CFH^+] = k_{-2}[OFH^+] \]  \hspace{1cm} (S-8a)

\[ [CFH^+] = \frac{k_{-2}[OFH^+]}{k_2} \]  \hspace{1cm} (S-8b)
Substituting S-8b into S-7b gives:

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

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

1/K₁ is essentially the acid dissociation constant Kₐ, so:

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

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

\[ pH = pK_a + \log \left( \frac{k_2[CF]}{k_{-2}[O\text{FH}^+]} \right) \]  
(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 µM (1-5) or 0.077 µM (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 ($\lambda_{exc} = 535$ nm) spectra of compound 9 at 0.077 $\mu$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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