Electronic Supplementary Information for


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Figure 1S. Computation of time filter patterns corresponding to a long time of 3.77 ns (---) from TG-II anion fluorescence emission, a short time of 0.31 ns (—) from TG-II neutral fluorescence emission, corresponding to scattered light and background signal (—–).

Figure 2S. Different correlation analyses of fluorescence fluctuation traces from 2 nM TG-II at pH 6.50, in the presence of 1.15 mM sodium phosphate buffer. (■) cross correlation; (●) time-gated cross correlation; and (▲) FLCS. In each case, the line represents the best fit.
Table 1S. Results obtained from the fit of the equation 2 to the experimental data shown in figure 2S, where [C] is the TG-II concentration calculated from <N> in the correlation functions, f is the fraction of dark state corresponding to the neutral form of TG-II, and τ is the protonation time for the chemical equilibrium neutral-anion.

<table>
<thead>
<tr>
<th></th>
<th>Cross-correlation</th>
<th>Time-Gate</th>
<th>FLCS</th>
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<tbody>
<tr>
<td>[C] / nM</td>
<td>12.5</td>
<td>4.8</td>
<td>1.96</td>
</tr>
<tr>
<td>f</td>
<td>0.4208</td>
<td>0.4572</td>
<td>0.4336</td>
</tr>
<tr>
<td>τ / s</td>
<td>2.17 x 10^6</td>
<td>1.75 x 10^-6</td>
<td>1.66 x 10^-6</td>
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</table>

As it can be seen, the value recovered for the fluorophore concentration by using the FLCS methodology is consistent with the nominal experimental concentration.

Figure 3S. Fraction of the neutral state of TG-II in water solution (f) vs. pH value, obtained from fitting eq. (2) in main text to FLCS curves of fluorescence fluctuations traces from TG-II in aqueous solution in the absence of buffer. The solid line represents the best fit of the experimental data to the equilibrium equation $f = H^+ / (K_a + H^+)$.
Figure 4S. Normalized lifetime filtered AC curves from TG-II solutions at different lithium phosphate buffer concentration: 0.25 mM (—), 0.50 mM (—), 1.00 mM (—), 1.50 mM (—) and 2.00 mM (—) at pH value 6.50.

Figure 5S. Normalized lifetime filtered AC curves from TG-II solutions at different caesium phosphate buffer concentration: 0.50 mM (—), 1.00 mM (—), 1.50 mM (—) and 2.00 mM (—) at pH value 5.80.
Figure 6S. Normalized lifetime filtered AC curves from TG-II solutions at different acetate buffer concentration: 0.174 mM (—), 0.390 mM (—), 0.711 mM (—) and 1.05 mM (—) at pH value 6.00

Figure 7S. Normalized lifetime filtered AC curves from TG-II solutions at different Tris buffer concentration: 0.06 mM (—), 2.38 mM (—), 6.23 mM (—) and 8.13 (—) at pH value 6.00