Supporting Information

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A close look at fluorescence quenching of organic dyes by tryptophan

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Supporting Information

Steady-state and time-resolved fluorescence and FCS data for ATTO 655, steady-state and time-resolved fluorescence and FCS data for rhodamine 6G, table with parameters of quenching interaction between Trp and MR121/ATTO 655/R6G.
Figure S1

(a) steady-state quenching
static quenching
static quenching corrected for sphere-of-action contribution.

(b) 

(c) 

(d) 

(e) 

(f) 

(g) 

(h)
Figure S1

(a,b) Steady-state (a) and time-resolved (b) bimolecular Stern Volmer plots of ATTO 655 (<1 µM) with Trp (0-50 mM) in PBS, pH 7.4. \( \tau_0 \) and \( I_0 \) are the fluorescence lifetime and intensity in the absence of Trp, \( \tau \) and \( I \) are the fluorescence lifetime and intensity in the presence of Trp. a) Squares represent the absolute intensity values without any correction. Circles give the intensity corrected for dynamic quenching, i.e. \( \frac{I}{I_0}/(\tau/\tau_0) = 1 + 6.0 \ M^{-1}\cdot[Trp] \) (as shown in (b)). Triangles give the corrected fluorescence intensity ratios divided by a sphere-of-action contribution \( \exp(\lambda[Trp]) \) with \( \lambda = (19 \pm 1) \ M^{-1} \). A linear fit of the corrected static quenching curve yields \( I_0/I_{corr} = 1 + 108 \ M^{-1}\cdot[Trp] \). The combination of sphere-of-action contribution and static quenching with a bimolecular association constant \( K_S = (108 \pm 1) \ M^{-1} \) are a measure of the stability of nonfluorescent complexes.

(c) Van’t Hoff plots of static quenching with (open circles) and without (black squares) correction for temperature dependent sphere-of-action contribution. Measurements were performed in PBS, pH 7.4, at 17 mM Trp. Linear fits of the data yield \( \ln K_S = I + m \ T^{-1} \) with \( I = (-2.2 \pm 0.1) \ M^{-1} \) and \( m = (2180 \pm 20) \ M^{-1} \ K^{-1} \) neglecting the sphere-of-action contribution, and \( I = (-3.7 \pm 0.1) \ M^{-1} \) and \( m = (2520 \pm 20) \ M^{-1} \ K^{-1} \) considering the sphere-of-action contribution.

(d) Absorption spectra (relative extinction \( \varepsilon \), in units of optical density, versus wavelength, \( \lambda \), in nm) of ATTO 655 (<1 µM resulting in optical densities of ~0.1 at the maximum extinction) measured in PBS, pH 7.4, with increasing Trp concentrations (0-50 mM). The appearance of isosbestic points at 531 and 667 nm indicates the formation of 1:1 complexes for Trp concentrations < 20 mM.

(e,f,g,h) Formation of nonfluorescent ATTO 655-Trp complexes monitored by FCS. (e) FCS was performed at room temperature in aqueous solution of ATTO 655 (~1 nM) and varying Trp concentrations (0-50 mM). Association- and dissociation rate constants \( k_{on} \) were derived from parameters fitted according to equation (12). The inset shows the inverse rate constants plotted as a function of Trp concentration. (f) Temperature dependence of association and dissociation rate constants measured by FCS.
Thermal destabilization as well as increasing diffusion coefficients causes a decrease in association and more pronounced in dissociation times (Inset). (g) The logarithm of either rate constant and (h) of the concentration-normalized correlation amplitude (being the ratio of association and dissociation rate constant divided by Trp concentration) follow an Arrhenius temperature dependence. Linear fits of Arrhenius plots yield: \( \ln(k_+)= (15\pm1)-(1400\pm350)T^{-1} \); \( \ln(k_-)=(22\pm1)-(3800\pm200)T^{-1} \); \( \ln(K^{0}_{\text{equ}})=(-8\pm1)+(2400\pm200)T^{-1} \). All errors are estimated from the fitting procedure and reflect the fact that correlations approach the lower time limit of the correlation measurement.

**Figure S2**

![Graphs showing various data points and trends related to the thermal destabilization and diffusion coefficients.](image-url)
Figure S2

(a,b) Steady-state (a) and time-resolved (b) bimolecular Stern Volmer plots of R6G (<1 µM) with Trp (0-50 mM) in PBS, pH 7.4. $\tau_0$ and $I_0$ are the fluorescence lifetime and intensity in the absence of Trp, $\tau$ and $I$ are the fluorescence lifetime and intensity in the presence of Trp. a) Squares represent the absolute intensity values without any correction. Circles give the intensity corrected for dynamic quenching, i.e. $(I_0/I)/(\tau_0/\tau)$ with $\tau_0/\tau = 1 + 10.6 M^{-1} \cdot [Trp]$. Triangles give the corrected fluorescence intensity ratios divided by a sphere-of-action contribution $\exp(\lambda[Trp])$ with $\lambda = (5 \pm 1) M^{-1}$. A linear fit of the corrected static quenching curve yields $I_0/I_{corr} = 1 + 19 M^{-1} \cdot [Trp]$. The combination of sphere-of-action contribution and static quenching with a bimolecular association constant $K_s = (19 \pm 1) M^{-1}$ are a measure of the stability of nonfluorescent complexes.

(c) Van’t Hoff plots of static quenching with (open circles) and without (black squares) correction for temperature dependent sphere-of-action contribution. Measurements were performed in PBS, pH 7.4 at 17 mM Trp. Linear fits of the data yield $\ln K_s = I + m T^{-1}$ with $I = (-1.9 \pm 0.1) M^{-1}$ and $m = (1630 \pm 20) M^{-1} K^{-1}$ neglecting the very small sphere-of-action contribution.

(d,e) Formation of nonfluorescent R6G-Trp complexes monitored by FCS. (d) FCS was performed at room temperature in aqueous solution of R6G (~1 nM) and varying Trp concentrations (0-50 mM). Association- and dissociation rate constants $k_{+/-}$ were derived from parameters fitted according to equation (12). The inset shows the inverse rate constants plotted as a function of Trp concentration. (e) Temperature dependence of association and dissociation rate constants measured by FCS. Due to weaker binding observed time constants are too small to reliably fit the data.
Table S1: Parameters of interaction between Trp and MR121, ATTO 655, R6G.

<table>
<thead>
<tr>
<th>Dye</th>
<th>$\tau_0$</th>
<th>$k_d$</th>
<th>$K_s$</th>
<th>$\lambda$</th>
<th>$\Delta G^{(1)#}$</th>
<th>$\Delta H^{(2)}$</th>
<th>$\Delta H^{+^{(2)}}$</th>
</tr>
</thead>
<tbody>
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<td>MR121</td>
<td>1.82</td>
<td>4.0±0.1</td>
<td>60.7±2</td>
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<td>29±3</td>
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<td>Atto 655</td>
<td>1.80</td>
<td>3.3±0.1</td>
<td>108±2</td>
<td>19±1</td>
<td>18.1±0.2</td>
<td>20.9±0.2</td>
<td>32±2</td>
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<tr>
<td>R6G</td>
<td>3.88</td>
<td>2.7±0.1</td>
<td>19±2</td>
<td>5±1</td>
<td>13.5±0.1</td>
<td>N.A.</td>
<td>N.A.</td>
</tr>
</tbody>
</table>

* measured at 22°C, (1) from ensemble van't Hoff plots, § (#) without (with) considering sphere-of-action contribution, (2) from FCS Arrhenius plots