Comparison of fluorescence decays for different maximum number of counts. The initial experiments in this project used a hydrogen-filled nanosecond flash lamp, which was the only excitation source available at the time. Decays with 2,000 counts in the channel of maximum intensity led to adequate statistics for the fits of the decays. Key experiments were repeated with the light emitting diode for which data accumulation is much faster and 10,000 counts can be accumulated in the channel of maximum intensity. The lifetimes recovered were the same with both number of counts. For example, for the emission of 2 µM Py in the presence of 30 mM NaCh a lifetime of 322 ns ($\chi^2 = 1.092$) was determined with the accumulation of 2,000 counts using the nanosecond flash lamp and a value of 323 ns ($\chi^2 = 1.019$) was determined for an accumulation of 10,000 counts with the light emitting diode. The Py lifetimes in the presence of 30 mM NaCh and 1.0 M NaCl were 380 ns ($\chi^2 = 0.951$) for an accumulation of 2,000 counts with the nanosecond flash lamp and 378 ns ($\chi^2 = 1.055$) for an accumulation of 10,000 counts with the light emitting diode.

Bile salts can contain a small amount of a fluorescence impurity, which varies from batch to batch. Attempts to remove this impurity through crystallizations were unsuccessful. This impurity emission has a short lifetime as measured in control experiments when the fluorophore was not present in solution. The lifetime for the impurity for long total time scales is defined by the width of each individual collection channel (e.g. 1.953 ns/channel in Fig. S1). The value for this fast decay is fixed to the value determined in control experiments for a particular total time scale, e.g. 5 ns in Fig S1 for a total time scale of 2 μs.

**Limiting anisotropy of perylene**

The limiting anisotropy of Pe ($r_0$) was evaluated from anisotropy decays in viscous solvents at low temperatures (near the freezing point of each solvent). The $G$ factor determined using the cryostat was 1.13 ± 0.01. For propylene glycol at -50 °C, the anisotropy did not decay much during the time window of the experiment (50 ns). In this case the spherical model was assumed (eq. S1, $i = 1$, where $\beta_i$ is the pre-exponential factor for each correlation time) for the fit of the intensity decays to equations 6 and 7 in the manuscript (note: eq. S1, $i = 1$ is
equivalent to eq. 8 in the manuscript). The recovered initial anisotropy for this solvent was 0.375 (Table S1), agreeing with the limiting anisotropy reported in the literature. Slightly lower values were obtained in ethylene glycol and glycerol (Table S1). The anisotropy decays in ethylene glycol and glycerol were fit using an ellipsoidal rotor model (eq. S1, i = 3) because the function $r(t)$ decayed over 50 ns.

$$r(t) = r_0 \sum_{i}^{i} \beta_i e^{-t/\phi_i}$$

(S1)

Table S1. Lifetimes determined with the emission polarizer at the magic angle ($\tau$) and initial anisotropies ($r_0$) for perylene ($3 \mu M$) in viscous solvents at low temperatures.

<table>
<thead>
<tr>
<th>Solvent (T / °C)</th>
<th>$\tau$ / ns</th>
<th>$r_0$</th>
<th>Global $\chi^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycerol (25)</td>
<td>4.69</td>
<td>0.337</td>
<td>1.19</td>
</tr>
<tr>
<td>Ethylene glycol (-8)</td>
<td>4.83</td>
<td>0.353</td>
<td>1.00</td>
</tr>
<tr>
<td>Propylene glycol (-50)</td>
<td>4.63</td>
<td>0.375</td>
<td>1.05</td>
</tr>
</tbody>
</table>

a, The anisotropy data was fit using an ellipsoidal rotor model (eq. S1, i = 3), where the $G$ factor and the fluorescence lifetime were fixed for the analysis. b, The decay was fit using the spherical rotor model (eq. S1, i = 1).

Effect of NaCl addition on the Py I/III ratios and lifetimes in the presence of 10 mM NaCh.

When Py ($2 \mu M$) was used as a guest in the presence of 10 mM NaCh, the fluorescence decays showed two components. One component was previously assigned to the lifetime of Py in water, while the second component corresponds to the lifetime of Py in the aggregate. The lifetime for the shorter-lived component was fixed to the lifetime of Py in water (127 ns). The lifetime of the long-lived component (Py in NaCh aggregates) became longer with the addition of NaCl (Table S2). The pre-exponential factor for the long-lived component, which is related to the concentration of bound Py, increased with the addition of NaCl, suggesting a higher degree of solubilization of Py into the NaCh aggregates. When the fluorescence spectra of the samples were analyzed, a decrease in the I/III ratio was observed (Table S2). This decrease was primarily caused by the increasing amount of Py incorporated into NaCh aggregates, as can be observed from the increase in the pre-exponential factor for the long-lived component (Table S2). In the absence of NaCl, the I/III ratio is closer to the ratio observed for Py in water (1.71), whereas in the presence of NaCl the I/III ratio is closer to the value for Py in NaCh aggregates (0.70-0.84).

Table S2. Fluorescence lifetimes ($\tau$), pre-exponential factors ($A$) and I/III ratios for Py ($2 \mu M$) in the presence of 10 mM NaCh at different concentrations of NaCl.

<table>
<thead>
<tr>
<th>[NaCl] / M</th>
<th>$\tau_1$ / ns ($A_1$)</th>
<th>$\tau_2$ / ns ($A_2$)</th>
<th>I/III ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>190 ± 8 (0.36 ± 0.06)</td>
<td>127 (0.65 ± 0.06)</td>
<td>1.617 ± 0.003</td>
</tr>
<tr>
<td>0.2</td>
<td>336 ± 3 (0.85 ± 0.04)</td>
<td>127 (0.16 ± 0.04)</td>
<td>0.903 ± 0.001</td>
</tr>
<tr>
<td>0.5</td>
<td>360 ± 1 (0.96 ± 0.01)</td>
<td>127 (0.04 ± 0.01)</td>
<td>0.768 ± 0.001</td>
</tr>
<tr>
<td>1.0</td>
<td>373 ± 5 (0.98 ± 0.01)</td>
<td>127 (0.03 ± 0.01)</td>
<td>0.719 ± 0.002</td>
</tr>
</tbody>
</table>

a, The data represents the average of two independent measurements; errors correspond to average errors or to the propagation of errors, whichever is greater. The fluorescence decays were fit to the sum of two exponentials, where the lifetime of Py in water was fixed to 127 ns.
Py fluorescence spectra at different excitation wavelengths.
The Py concentration (2 µM) used for the measurements in the presence of NaCh is higher than the Py concentration soluble in water (0.6 µM). A control experiment was performed in which a 2 µM Py/30 mM NaCh solution was excited at different wavelengths. The normalized spectra had the same shape (Fig. S2) showing that only monomeric Py was present.

![Figure S2](image)

**Figure S2.** Normalized emission spectra at 382 nm for Py (2 µM) in the presence of 30 mM NaCh when the solution was excited at 331 nm (black), 335 nm (red) and 340 nm (blue). The noise level is larger for the longest excitation wavelength because the overall intensity is much lower.

Quenching by nitromethane or iodide anions of the singlet excited state of Py in water and in the presence of NaCh.
In the absence of NaCl the fluorescence decay of Py in the presence of 30 mM NaCh and nitromethane was not adequately fit to a sum of two exponentials, where one lifetime corresponded to the impurity emission of bile salt and the second lifetime corresponded to the emission of pyrene. The decays were fit to the sum of three exponentials, where Py had a short- and a long-lived component (see decay Fig. S1). The quenching efficiency for both Py components is less efficient than in water ($k_q = 7.1 \times 10^9$ M$^{-1}$ s$^{-1}$), where the $k_q$ value was $(2.1 \pm 0.3) \times 10^9$ M$^{-1}$ s$^{-1}$ for the short-lived Py species and $(8.4 \pm 0.2) \times 10^8$ M$^{-1}$ s$^{-1}$ for the long-lived Py species. These quenching experiment show that the short-lived Py species does not correspond to Py in water because the excited state of Py is protected from quenching. However, the higher $k_q$ value than that observed for the long-lived component suggest that the Py-NaCh complex is different from the major aggregate present. The fact that this minor Py-NaCh component was not observed in the quenching experiment by iodide anions suggests that the complex is negatively charged and efficiently repels the anionic quencher.

![Figure S3](image)

**Figure S3.** Quenching plot for the quenching with nitromethane of Py (0.5 µM) in water (simulated data based on $k_q = 7.1 \times 10^9$ M$^{-1}$ s$^{-1}$, black solid line) and Py (2.0 µM) in the presence of 30 mM NaCh (●, blue - short lifetime and ○, red - long lifetime).

The quenching plots for the Py fluorescence in the presence of 30 mM NaCh at various concentrations of NaCl were linear (Fig. S4 and S5).

![Figure S4](image)

**Figure S4.** Quenching plot for the quenching by nitromethane of the fluorescence of Py (2 µM) in the presence of 30 mM NaCh and increasing concentrations of NaCl: 0 M (●, long lifetime), 0.2 M (△) and 1.0 M (□).
Figure S5. Quenching plot for the quenching by iodide anions of the fluorescence of Py (2 \(\mu\)M) in the presence of 30 mM NaCh and increasing concentrations of NaCl: 0 M (\(\bullet\)), 0.2 M (\(\Delta\)) and 1.0 M (\(\square\)).

**Time-resolved anisotropy studies in glycerol/water mixtures.**

The dependence of the measured correlation time with the viscosity of the solution at constant temperature was studied in glycerol/water mixtures. The proportion of glycerol was increased from 0 to 30% w/w for a solution containing perylene (3 \(\mu\)M), NaCh (30 mM) and NaCl (0.2 M). The viscosities of water/glycerol mixtures were taken from the literature at 20 °C.\(^4\) The correlation time increased as the viscosity increased but the relationship is not as linear as observed for the dependence of the correlation time with \(\eta/T\) (see Fig. 4 in the paper). The deviation from linearity could be due to the presence of a high concentration of glycerol, which could change the structure and size of the aggregates. A previous study suggested that ethylene glycol makes the bile salts aggregates more flexible and/or smaller, altering the binding dynamics of the guests.\(^5\)

References