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Water Soluble Cruciforms: Effect of Surfactants on Fluorescence

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Abstract: Three 1,4-bis-(aminostyryl)-2,5-bis(phenylethynyl) benzenes carrying four, six, or eight acetic acid units were investigated for their surfactochromicity. Anionic, cationic, and nonionic surfactants as well as a surfactant-like protein (bovine serum albumin, BSA) were added to buffered solutions of the XFs in water, causing—in most cases—the fluorescence quantum yield to increase significantly and a blue- or red-shifted emission to be observed. The addition of cationic (cetyltrimethylammonium bromide, CTAB) and neutral (Brij 35, TWEEN 20 and Triton X-100) surfactants to XFs causes a red shift in their emission at low or very low surfactant concentrations that we attribute to surfactant-induced excimer formation. The fluorescence quantum yield is in most cases a monotonous function of surfactant concentration. For the investigated ionic surfactants, the fluorescence quantum yield of the XFs does not change much after the critical micelle concentration (CMC) of the surface is reached. However, in the case of nonionic surfactants, the fluorescence quantum yields of the XFs starts to increase after the CMC has been reached, suggesting that different effects are involved.

Keywords: cruciforms · fluorescence · materials science · sensors · surfactants

Introduction

Herein, we describe the effect of anionic, cationic, and neutral surfactants on the fluorescence wavelength and fluorescence intensity of **1–3** in aqueous buffered solutions. We see significant (**3**) or large increases in the fluorescence intensity and either red or blue shifts of the emission. Functional water-soluble fluorophores that carry sensory appendages are popular for the detection of pools of free (i.e., not tightly bound) metal cations in cells and cell compartments.[1] They are also important as environmental probes.[2] Metals of interest in the biological realm are calcium,[3] magnesium,[4] zinc,[5] aluminum,[6] and copper.[7] Alzheimer's[8] is possibly connected to an enhanced concentration of zinc ions in brain tissue.[9] Generation, function, and fate of free metal ion pools are of fundamental interest. A popular motif for metal binding is the APTRA-module,[10] displayed in the structural formula of **2**. We are interested in the synthesis and property evaluation of responsive fluorophores, with action modes based on the spatial separation of frontier molecular orbitals (FMO).[11,12,13] Such fluorophores are composed of a molecule featuring two axes connected by a benzene ring and termed cruciforms (XF, 1,4-distyryl-2,5-bis(phenylethynyl)benzenes). The spatial separation of the FMOs is dependant upon the substituent pattern on the axes and particularly distinctive if one axis carries donor substituents, while the other transverse is substituted by acceptors. Examples of other fluorophores that also display space separated FMO patterns are Haley's 1,2,4,5-tetrakis(arylethynyl)benzenes,[14] Scherf's swivel-cruciforms,[15] Diederich's tetraethynylethylenes,[16] Marks' X-molecules,[17] and Fahrni's pyrazolines.[18] We investigated the fundamental photophysics of XFs such as **4** in non-aqueous solvents, where they display high quantum yields and significant shifts upon exposure towards divalent main and transition metal triflates. XFs **1–3** were reported and their photophysics in water and in the presence of metal cations examined.[19] While these XFs did show some metal sensitivity in water, the mechanism of the sensing action was the break-up of excimeric aggregates rather than a specific color response by coordination of the metal cations to the aniline nitrogens. We found also that the XFs **1–3** display modest (**3**, $\Phi = 4.4\,\%$) to vanishing (**2**) quantum yields in water, even when investigating these XFs in dilute solution; part of the low quantum yield is the coupling of overtone vibrations of water to the excited state of the XFs, leading to their effi-

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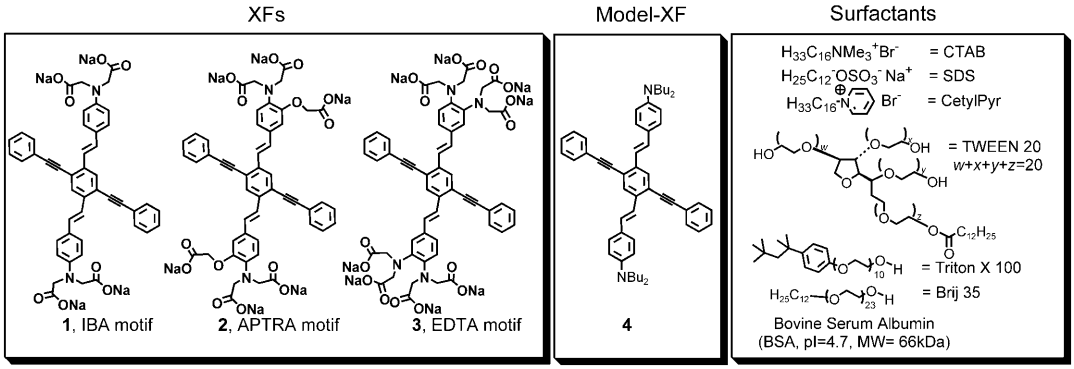

cient deactivation.[20] Changing the solvent to D_2O led to a two- to fourfold increase in emission efficiency but still, the quantum yields of the XFs were low.

Hydrophobicities and hydrophilicities in cellular compartments differ from those of buffers; embedded chromophores may experience a modulation of their fluorescence efficiency. A classic example is diphenylhexatriene, which is almost non-fluorescent in solution, but becomes emissive upon insertion into the rigid environment of a membrane.[21] Other fluorescent dyes display similar behavior and have been employed to probe the phase properties of some proteins.[22] As a consequence, fluorescent water-soluble probes that display polarity and phase dependent properties are of interest. The simplest way to test if a fluorophore might be promising for such applications is to evaluate their surfactochromicity, that is, the change of their optical properties when exposed to different surfactants.[23] The XFs **1–3** display significant effects when dissolved in surfactant solutions and give surprising concentration-dependant effects, a good number of which are not linked to the critical micelle concentration (CMC) of the investigated surfactants.

Results and Discussion

In a first experiment, we prepared phosphate buffered (pH 7.0, 0.1 mol L^{-1}) solutions of **1–3** (5 μmol L^{-1}), added an excess of surfactant, and obtained a photograph (Figure 1).

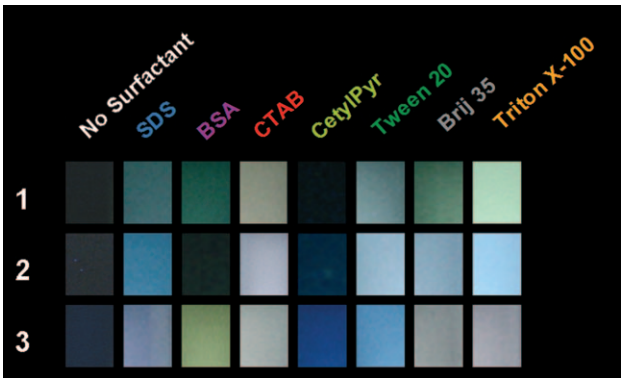

Figure 1. Exposure of solutions (5 μmol L^{-1}) of **1–3** towards different surfactants (10 mmol L^{-1}; SDS: 100 mmol L^{-1}). BSA: Bovine serum albumin.

The fluorescence of the XFs increases by the addition of the surfactants and a wavelength shift in emission is observed. The UV/Vis spectra of the XFs (see Supporting Information) show a slight red shift of the absorption maximum and an increase in the intensity of the secondary band around 400 nm (Table 1). Figure 2 displays the normalized emission spectra. The largest shifts were observed for **1**, where the addition of SDS led to an almost 100 nm blue shift in emission. In the case of **2**, we also observe a blue shift, but it is much less distinct. In **3**, only a red shift upon exposure to surfactants is observed. The surfactant cetylpyridinium bromide (CetylPyr) leads to quenching of the fluorescence in

Table 1. Optical properties of XFs **1–3** upon exposure towards different surfactants (10 mmol L^{-1} concentration of surfactant; SDS: 100 mmol L^{-1}).

	1			**2**			**3**		
	Absorption λ_{max} [nm]	Emission λ_{max} [nm]	Φ[a]	Absorption λ_{max} [nm]	Emission λ_{max} [nm]	Φ	Absorption λ_{max} [nm]	Emission λ_{max} [nm]	Φ
CTAB	343, 404	536	0.118	347	470	0.211	331	462	0.317
Cet-Pyr	333	–	q[b]	343	–	q[b]	331	450	0.068
SDS	329	465	0.034	330	463	0.122	327	454	0.210
Triton X-100	336, 401	521	0.178	340	469	0.422	335	455	0.348
Brij 35	329	466, 526	0.155	335	468	0.238	332	460	0.401
Tween 20	329	516	0.179	338	470	0.340	332	460	0.392
BSA	329	515	0.028	330	–	q[b]	327	498	0.078
None	331	546	0.008	328	485	q[b]	324	437	0.044

[a] Quantum yields were measured using quinine sulfate solution in H_2SO_4 as standard, [b] quenched solutions, $\Phi \approx < 0.003$.

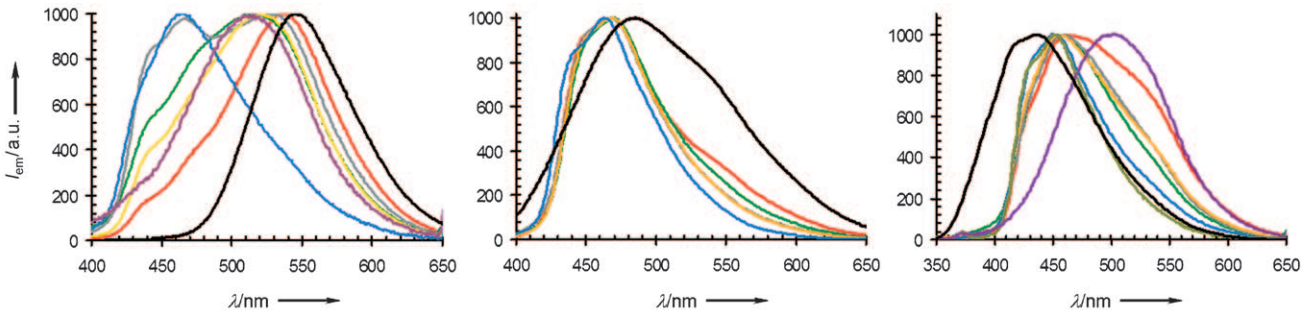

Figure 2. Normalized emission spectra of the XFs **1–3** (5 μmol L^{-1} from left to right) upon exposure towards different surfactants (10 mmol L^{-1}; SDS: 100 mmol L^{-1}). Color coding is identical to that shown in Figure 1 (phosphate buffer, pH 7.0, 0.1 mol L^{-1}).

1–3, arising from efficient excited state electron transfer from the XF to the pyridinium salt. This is not surprising, as related paraquat-based species are also powerful fluorescence quenchers.[24,25]

The three XFs, even though they share the same carbon skeleton and similar functionalities, respond differently to the addition of each surfactant. While Figures 1 and 2 display the qualitative changes that arise upon addition of surfactants to the fluorophores **1–3**, Figure 3 displays the absolute quantum yields of **1–3** upon addition of an excess of the surfactant. While the quantum yields for the XF alone are low, the addition of surfactants to **2** causes a more than 1000-fold increase of the fluorescence quantum yield. The most efficient enhancers are the three non-ionic surfactants Tween 20, Brij 35, and Triton X-100, with Triton being most efficient for **2** and Brij most efficient for **3**. In the case of **1**, all non-ionic surfactants and CTAB work well, while SDS and the protein bovine serum albumin (BSA), a negatively charged biological surfactant, exhibit lesser effects on **1**. In the case of **2** and **3**, BSA has only a small effect on the fluorescence quantum yields.

We investigated the concentration dependence of the surfactants upon the absorption and emission of the XFs **1–3**. Our initial hypothesis was that the largest changes in emission wavelength and emission quantum yield would coincide with the known critical micelle concentrations (CMC) of the different surfactants. However, as Figures 4 and 5 display, this is decidedly not the case.

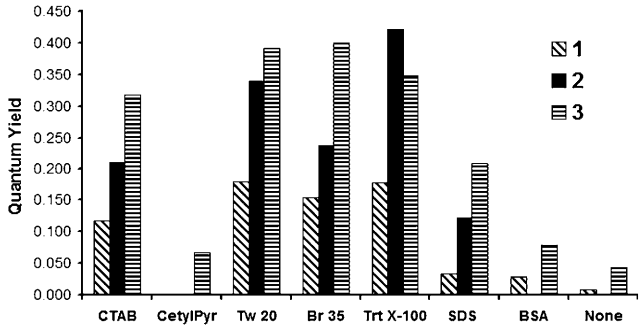

Figure 3. Quantum yields of **1–3** upon addition of an excess (10 mmol L^{-1}; SDS: 100 mmol L^{-1}) of surfactants to their aqueous solution (phosphate buffer, pH 7.0, 0.1 mol L^{-1}).

Charged Surfactants

We will discuss the influence of the two charged (SDS and CTAB) surfactants on the absorption and fluorescence of **1–3** first. Changes in absorption take place below the CMC; absorption maxima are invariable above the CMC. This is a general behavior for **1–3** in the presence of SDS (Figure 6, middle). In the case of CTAB there is a unique red shift of the maxima for **1** and **3** at low concentrations of surfactant, suggesting an electrostatic interaction between the surfactant and the XFs (positively and negatively charged, respectively) in which the formation of a micelle is not necessary (Figure 6, left). This is not the case for **2**, where a continuous red shift occurs; it is more pronounced than in **1** and **3**, and only at concentrations above the CMC is the emission maximum reached.

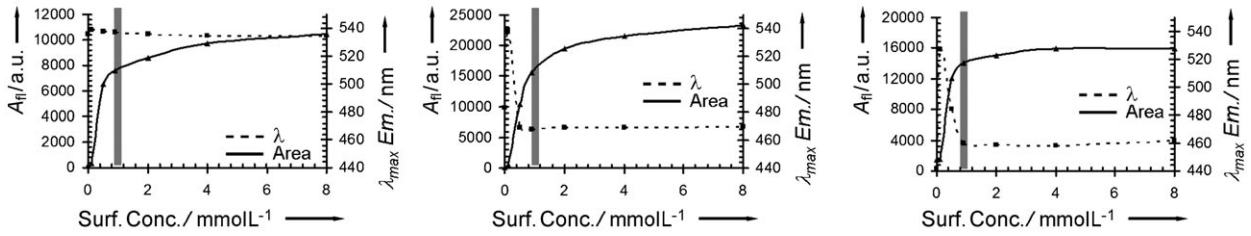

Figure 4. Effect of the addition of CTAB upon the fluorescence quantum yield (solid) and wavelength (dotted) of **1–3** (5 μmol L^{-1}). In Figures 4 and 5, the XF **1** is shown on the left hand side, **2** in the middle and **3** on the right hand side (phosphate buffer, pH 7.0, 0.1 mol L^{-1}). CMC of CTAB (gray area): 0.92–1 mM.

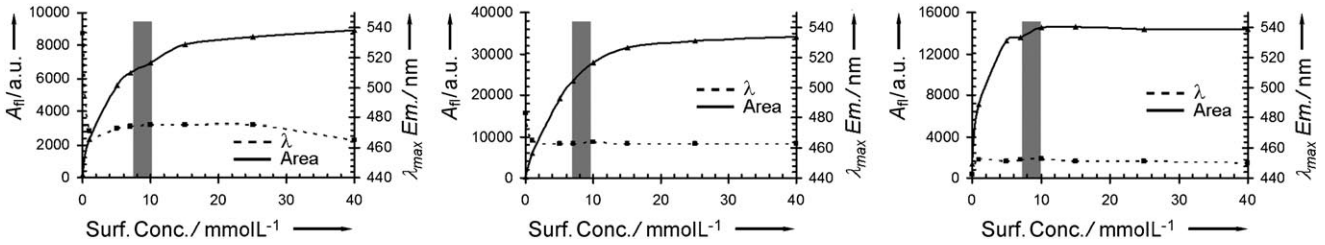

Figure 5. Effect of the addition of SDS upon the fluorescence quantum yield (solid) and wavelength (dotted) of **1–3** (5 μmol L^{-1}, phosphate buffer, pH 7.0, 0.1 mol L^{-1}). CMC of SDS (gray area): 7–10 mM.

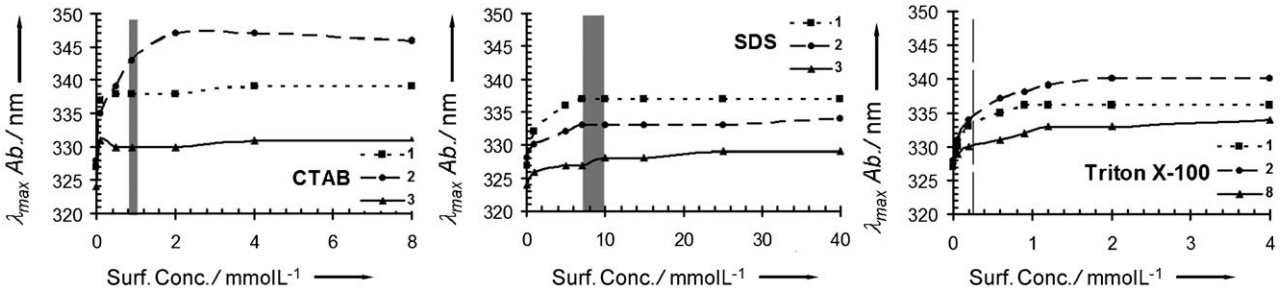

Figure 6. Effect of the addition of negatively, positively and uncharged surfactants upon the wavelength of maximum absorption of **1–3** (5 μmol L^{-1}, phosphate buffer, pH 7.0, 0.1 mol L^{-1}; graphics of the spectra are presented in the Supporting Information).

If one looks at the fluorescence intensity of **1–3**, at the CMC of CTAB or SDS, an inflection point is reached, suggesting that the mechanism of fluorescence enhancement changes at that point (Figure 4). Above the CMC the gain in fluorescence intensity flattens, that is, once the CMC is reached, most of the fluorescence enhancement is realized. Increase of surfactant concentration beyond the CMC only leads to a small additional increase in fluorescence intensity. A red shift of the emission wavelength of the XFs is observed at very low surfactant concentrations for CTAB (1:20 molar ratio of XF to surfactant) and a blue shift is observed for SDS (1:200 molar ratio of XF to surfactant). This is notable as it happens considerably below the CMC. In the case of CTAB (Figure 4) one can speculate that electrostatic complexes (1:8, 1:6, and 1:4 for **1**, **2**, and **3**, respectively) of XFs and surfactant form. These would show excimeric emission or experience planarization of the π-system including the aniline groups in the excited state, leading to a strong red shift for **2** and **3**. These two XFs are probably not in a planar conformation in the ground state, arising from steric, electronic, and electrostatic effects. The sterically less encumbered XF **1** does not experience deplanarization in the ground state according to our previously reported results.[19]

The CMC of CTAB only plays a minor role in the variation of the emission wavelength of the XFs (Figure 7). After the addition of very small amounts of CTAB, emission at 530–540 nm is observed for **1–3**. This emission data is close to that obtained for **4** in dichloromethane or chloroform (519 nm). The emission is considerably red shifted from that of the three ethyl-ester-derivatives of **1–3**, which emit at 486–504 nm in chloroform solution. The red shift could arise for several reasons: 1) formation of an excimeric species upon complexation, 2) detergent-induced planarization of the XFs, and 3) additional red shift arising from the increased polarity of water when compared to that of dichloromethane.

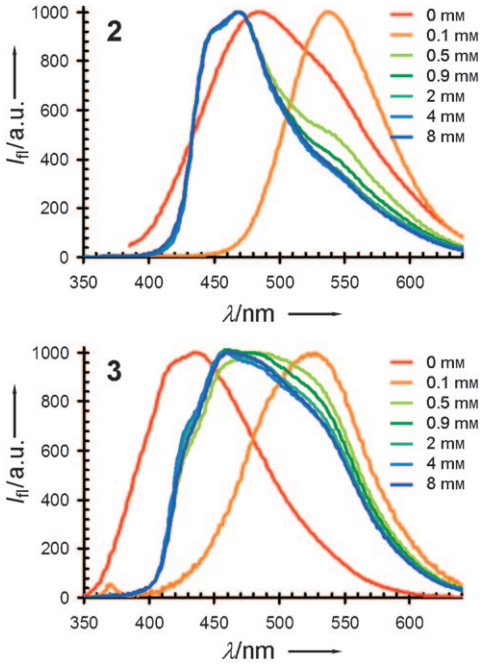

Figure 7. Normalized fluorescence of **2** and **3** upon the addition of CTAB (CMC = 1 mM). 5 μmol L^{-1} concentration of XF.

Upon increasing the CTAB concentration, the emission blue shifts back to 470 nm in **2** and to 450 nm in **3**. We assume that with an excess of CTAB present, a non-planar

and non-excimeric species forms in which the aniline unit is twisted with respect to the phenyl ring in the excited state. In addition, the environment is now less polar owing to the association of the XF skeleton with the hydrocarbon tail of CTAB.

In the case of SDS (Figure 5), for **1** and **2**, a blue shift of emission occurs at very low surfactant concentration despite the red-shifted absorption, while for **3**, a monotonous red shift occurs upon addition of SDS. In contrast to CTAB, after the addition of a small amount of SDS, the XF emission maximum does not shift anymore for any of the XFs. Increasing concentrations of SDS, however, leads to an increased fluorescence intensity until the CMC is reached. The XFs **1** and **2** might experience a twisting of the aniline nitrogen in the excited state upon addition of small amounts of SDS, which would lead to their blue-shifted emission spectra.[26]

Non-Ionic Surfactants

The non-ionic surfactants TWEEN, Brij, and Triton are derivatives of oligoethyleneglycol or polyethyleneglycol ethers featuring hydrophobic tails. They are structurally related and their influence on the fluorescence of the XFs is similar (Figure 8–10). The addition of these surfactants to solutions of **1–3** leads to an increase of the XFs fluorescence intensity far beyond the CMC of the surface. Below the CMC, only small changes were observed, opposite to the cases of SDS and CTAB. If Triton is added, **2** displays the most clear-cut responses connecting the CMC and emission wavelength. The emission of **2** is 540 nm (red shift of 80 nm) at the CMC of Triton (Figure 8). Above the CMC, the fluorescence intensity increases monotonically but the emission wavelength decreases again to 470 nm. In the case of **3**, the behavior is similar, while for **1**, there is only a blue shift at very low surfactant concentrations followed by a gradual red shift. Above 1 mmol L^{-1} concentration of Triton, the emission

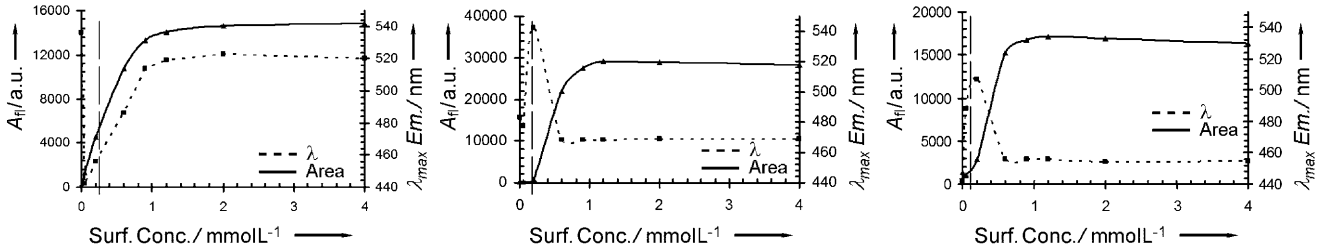

Figure 8. Effect of the addition of Triton X-100 upon the fluorescence quantum yield and wavelength of **1–3** (from left to right). (5 μmol L^{-1}, phosphate buffer, pH 7.0, 0.1 mol L^{-1}). CMC of Triton X-100 (vertical dotted line): 0.22-0.24 mM; Aggregation number (*N*) of Triton X-100 = 150.

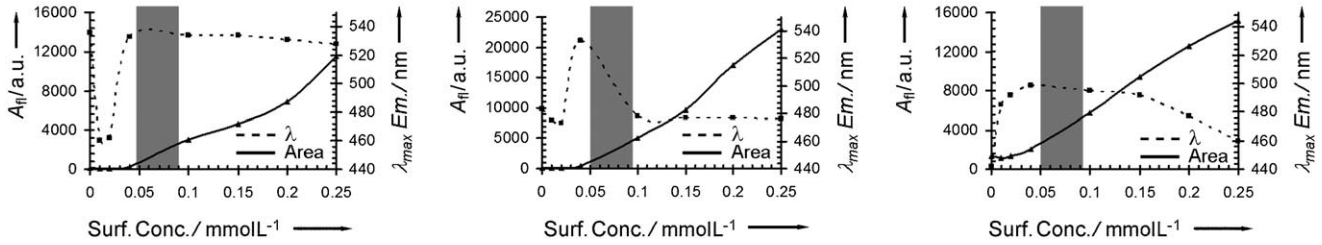

Figure 9. Effect of the addition of Brij 35 upon the fluorescence quantum yield and wavelength of **1–3** (5 μmol L^{-1}, phosphate buffer, pH 7.0, 0.1 mol L^{-1}). CMC of Brij 35 (gray area): 0.05–0.09 mM. Aggregation number (*N*) of Brij 35 = 65–69.

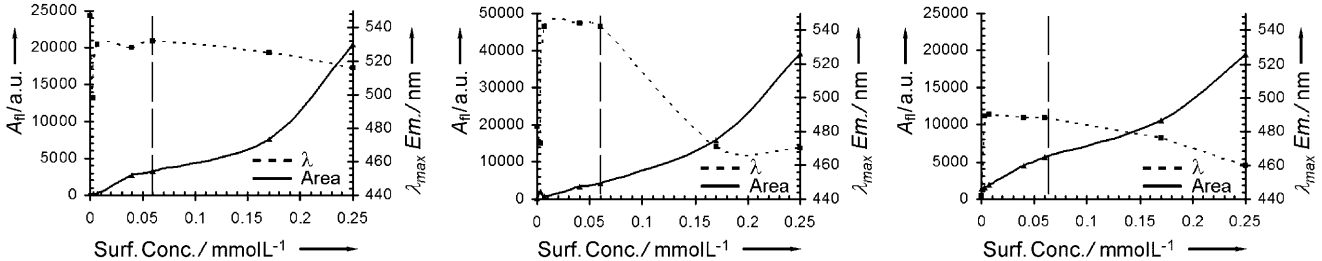

Figure 10. Effect of the addition of TWEEN 20 upon the fluorescence quantum yield and wavelength of **1–3** (5 μmol L^{-1}, phosphate buffer, pH 7.0, 0.1 mol L^{-1}). CMC of TWEEN 20 (vertical dotted line): 0.06 mM. Aggregation number (*N*) of TWEEN 20 = 70.

wavelength does not change anymore and no change in the emission intensity is observed.

In the case of Brij (Figure 9), the CMC is lower and the literature gives a CMC range. Despite the lack of a clear definition of the CMC, the effects on the emission of **2** and **3** are similar to those recorded for Triton, but the features appear over a narrower concentration range of surfactant. XF **1** is different; after a blue shift of the fluorescence at low surfactant concentration, the original band around 530 nm is recovered when the CMC is reached. In terms of fluorescence intensity, the behavior of **1** is similar to that of **2** and **3**.

If TWEEN is added to XFs **1–3**, more complex effects are observed. In **1**, after an initial blue shift, the addition of TWEEN up to the CMC leads to a re-red-shifted emission (Figure 10). Beyond the CMC, the emission wavelength starts to slightly blue shift again. If added to **2** and **3**, TWEEN leads to a significant red shift in emission at very low concentrations. This shift of the emission wavelength is somewhat unexpected as there are no easily identified intermolecular forces that would be responsible for the shift in emission wavelength. The gross overall patterns for TWEEN are similar to those observed for the addition of Triton and Brij, first red shifting of the XF′s emission and after reaching the CMC of the surface, blue-shifting to the starting wavelength values. In the case of Brij and Triton the effects are more pronounced and easier to interpret as they coincide with their CMC.

What is the reason for this striking red shifted but weak emission? An explanation would be the excimeric, micelle-constrained species formed from **2** or **3** once the CMC of the non-ionic surfactants is reached. The concentration of micelles at the CMC, $[M_{CMC}]$, (not the concentration of the surfactant) is defined as $[M_{CMC}] = CMC/N$ with N being the number of surfactant molecules necessary to form a micelle (aggregation number); $N_{Triton} = 150$, $N_{Brij} = 67$ and $N_{TWEEN} = 70$. As a consequence, $[M_{CMC}]_{Triton} = 1.5$ μmol, $[M_{CMC}]_{Brij} = 1.0$ μmol, and $[M_{CMC}]_{TWEEN} = 0.9$ μmol,[27] and the ratio of $[XF]/[M_{CMC}]_{Triton} = 3.3$, $[XF]/[M_{CMC}]_{Brij} = 5$, and $[XF]/[M_{CMC}]_{TWEEN} = 6$. These ratios are approximate but they show nicely that at the CMC or in its vicinity, each micelle solubilizes several XF molecules. As the interior of the micelle is more hydrophobic and rigid than the surrounding aqueous solution, the spatially constrained XFs form excimers in the micelle. These display fluorescence, albeit with weak intensity. In the regimen where the concentration of the micelles is much larger than that of the XF, each XF occupies its "own" micelle. This rigid and hydrophobic environment turns on the fluorescence further but at the same time, leads to the disappearance of the excimers. The most clear cut examples are the XFs **2** and **3** in the presence of Triton or Brij. In the case of XF **1**, the aforementioned interpretation is not viable and we can only speculate what causes the distinct drop in the emission wavelength at low surfactant concentrations. However, for potential biological applications, the turn on of the fluorescence in a micellar environment is the most important property of the system.

Conclusions

The interaction between XFs **1–3** and surfactants leads to changes in their optical and luminescent behavior. A dramatic increase of the fluorescence quantum yield, especially for **2**, is observed. A red shift of the absorption maxima is also observed upon addition of surfactants. At the CMC, the ionic surfactants cause a significant gain in the emission intensity and beyond the CMC, any additional gain in emission intensity is small. Upon interacting with nonionic surfactants, the XFs' fluorescence intensity continues to increase even when the concentration of the added surfactant is $> 5 \times$ CMC. At the CMC of Triton and Brij, **2** and **3** display red-shifted emission perhaps arising from excimeric species. Red-shifted emission is also seen for CTAB but at much lower surfactant concentrations, probably as a result of complex formation between the positively charged CTAB and the negatively charged XFs **2** and **3**. The XFs **1–3**, display a low quantum yield in water, but a striking increase in quantum yield is observed when they are in confined compartments such as micelles. These might be of interest as "rinseless" (that is background free) dyes for rigid cell compartments such as the endoplasmic reticulum, cell membranes, and so forth that feature somewhat hydrophobic environments.[28]

Experimental Section

General

All chemicals were purchased from Aldrich Chemical, Acros, TCI America, or Fischer Scientific and used without purification unless otherwise specified. All pH measurements were made using a calibrated VWR SympHony SP20 digital pH meter. All absorption spectra were collected using a Shimadzu UV-2401PC spectrophotometer. The emission spectra of solutions were acquired using a Shimadzu RF-5301PC spectrofluorophotometer or a PTI QuantaMaster spectrofluorophotometer outfitted with a xenon arc lamp and series 814 PMT detector. Scattering peaks were removed by subtracting our fluorescence spectra without added fluorophores from all spectra. Quantum yields for cruciforms were measured using standard procedures.[29] In all cases, quinine sulfate was used as a standard and all solutions were purged with nitrogen prior to measurement. Photos were taken under blacklight ($\lambda_{ex} = 365$ nm) and photographed using a Canon EOS Digital Camera equiped with an EFS 18–55 mm lens.

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