

# Group 14 Metallafluorenes as Sensitive Luminescent Probes of Surfactants in Aqueous Solution

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## Research Article

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# Abstract

Sila- and germafluorenes containing alkynyl(aryl) substituents at the 2,7- position are strongly emissive with high quantum yields in organic solvents. Provided they are sufficiently soluble in water, their hydrophobic structures have the potential for many biological and industrial applications in the detection and characterization of lipophilic structures. To that end, the emission behaviors of previously synthesized 2,7- bis[alkynyl(biphenyl)]-9,9-diphenylsilafluorene (**1**), 2,7- bis[alkynyl(methoxynaphthyl)]-9,9-diphenylgermafluorene (**2**), 2,7- bis[alkynyl(*p*-tolyl)]-9,9-diphenylsilafluorene (**3**), and 2,7- bis[alkynyl(*m*-fluorophenyl)]-9,9-diphenylsilafluorene (**4**) were characterized in aqueous solution and in the presence of various surfactants. Despite a high degree of hydrophobicity, all of these metallafluorenes (MFs) are soluble in aqueous solution at low micromolar concentrations and luminesce in a common aqueous buffer. Further, the 2,7 substituent makes the emission behavior tunable (up to 20 nm). Fold emission enhancements in the presence of various surfactants are highest toward Triton X-100 and CTAB (ranging from 5–25 fold) and are lowest for the anionic surfactants SDS and SDBS. These enhancements are competitive with existing probes of surfactants. Quantum yields in buffer range from 0.11 to 0.34, competitive with many common fluorophores in biological use. Strikingly, MF quantum yields in the presence of TX-100 and CTAB approach 100% quantum efficiency. MF anisotropies are dramatically increased only in the presence of TX-100, CTAB, and CHAPS. Coupled with the above data, this suggests that MFs associate with neutral and charged surfactant aggregates. Interactions with the anionic surfactants are weaker and/or leave MFs solvent exposed. These properties make metallafluorenes competitive probes for surfactants and their properties and behaviors, and thus could also have important biological applications.

## 1. Introduction

Sila- and germa-fluorenes, also known as dibenzosiloles or dibenzogermoles, are a class of photoluminescent compounds with high quantum yields in organic solvents [1, 2]. Interest in their potential applications as OLEDs recently drove us to synthetically explore 2,7-alkynyl(aryl) substitutions (Fig. 1A), which could be exploited to tune spectral behavior by extending the high degree of conjugation [1, 2]. Such substitutions can also attenuate solubility in various solvents.

These optical properties and the hydrophobic nature of the structures suggest that if they are sufficiently soluble in aqueous solution, the utility of these compounds could be expanded to detect surfactants, common contaminants in wastewater [3–5], and could also have applications in the probing of lipid structures and behaviors [3–6].

There are a number of known fluorescent indicators of surfactants (Fig. 1B). They include the cationic pyridinium derivative [4-((1E,3E)-4-(4-(dimethylamino)phenyl)buta-1,3-dien-1-yl)-1-methylpyridin-1-ium (DABP)] [5]; 2,3-substituted naphthalimide dye derivatives DMN-Bu [7] and diethyl 6-(dimethylamino)naphthalene-2,3-dicarboxylate (DMNDC) [8]; the cationic polythiophene derivative poly[3-(1,1'-dimethyl-4-piperidinemethylene)thiophene-2,5-diyl chloride] (PDPMT-Cl) [9]; and N-n-octyl-4-(1-

methylpiperazine)-1,8-naphthalimide iodide [C<sub>8</sub>ndi]I [3]. The degree of characterization of the behaviors of these compounds varies widely, but a typical working concentration for these compounds is 10 μM [3, 5, 7]. Fold enhancements upon the addition of surfactants also vary, but typically they range from a few-fold to 10–20 fold [10, 7, 4].

Metallafluorenes (MFs) of sufficient solubility in aqueous solution and sensitivity to surfactants would represent a new class of fluorescent detectors for the detection and study of surfactants and relevant biological structures. Here we characterize the aqueous solution emission behaviors of four previously synthesized metallafluorenes [1, 2] and their interactions with a variety of surfactant solutions. Indeed, we demonstrate here that these MFs are soluble in aqueous solution at low μM concentrations and luminesce under these conditions with competitive quantum yields. Further, dramatic spectral changes, increases in quantum yield and anisotropies occur in the presence of some surfactants, which demonstrate their potential as fluorescent probes of lipophilic structures in aqueous environments.

## 2. Materials And Methods

### 2.1. Materials.

Coumarin-102 dye (99%), Triton X-100, sodium dodecylbenzenesulfonate (SDBS), and hexadecyltrimethylammonium bromide (C-TAB) were purchased from Sigma-Aldrich (St. Louis, MO). Dodecyl sulfate sodium salt (SDS) was purchased from Fisher Scientific (Waltham, MA). CHAPS was purchased from Anatrace (Maumee, OH) and 200 proof ethanol was purchased from Decon Labs (King of Prussia, PA). All chemicals used were of reagent grade and were used as received without further purification.

### 2.2. Preparation of Metallafluorenes.

Fluorescent 2,7-disubstituted sila- and germafluorenes **1–4** were synthesized as previously described using an appropriate ethynyl aryl precursor in a palladium-catalyzed Sonagashira cross-coupling reaction [1, 2].

### 2.3. Spectroscopy.

Absorbance spectra were recorded on a Shimadzu 1800 with slits set to 1 nm.

Luminescence experiments were performed in acid-washed quartz cuvettes at 10 mM Tris, pH 8.0 on a T-formatted Fluorolog-3 (SPEX) spectrofluorimeter equipped with a polarization assembly. The temperature was maintained 25°C with a thermostatted cell holder equipped with a magnetic stirrer. All intensities were repeated at least three times on different days and the results averaged.

For fluorescence anisotropy, at least three readings were collected over a 0.1 s integration time each and averaged. Anisotropy values were obtained in triplicate and automatically calculated from **Eq. 1**:

$$A = \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + 2I_{\perp}} \quad \text{Eq. 1}$$

where  $I$  is the recorded intensity at the indicated polarizer orientations and  $A$  is the anisotropy. This experiment was repeated at least three times on different days and the results averaged.

Emission spectra collected in the presence of salts ( $\text{Na}_2\text{HPO}_4$ ,  $\text{K}_2\text{HPO}_4$ ,  $\text{CaCl}_2$ ,  $\text{ZnCl}_2$ ,  $\text{NH}_4\text{Cl}$ ,  $(\text{NH}_4)_2\text{SO}_4$ ,  $\text{MgSO}_4$ ) were conducted at 1 mM salt (a common concentration used in the literature [6] and 1  $\mu\text{M}$  MF.  $\text{ZnCl}_2$  results in MF precipitation and therefore these data were not reported.

Quantum yield was measured using the previously reported relative method [11] at an excitation wavelength of 350 nm using a range of absorbances. Absorbances were kept below 0.1 to minimize non-linear effects [12]. The fluorescence spectra were recorded from 375 nm to 625 nm at the excitation wavelength of 350 nm. An excitation and emission slit width of 1.0 nm was used. The spectra of the reference standard and the unknown were measured under identical conditions. The slope of the integrated fluorescence intensity versus absorbance was used to calculate the quantum yield using Eq. 2. Coumarin 102 dye in ethanol, with a  $\Phi_f$  of 0.764 [13], was used as the reference standard for all determinations.

$$\phi_{\text{unknown}} = \phi_{\text{standard}} \left( \frac{m_{\text{unknown}}}{m_{\text{standard}}} \right) \left( \frac{n_{\text{unknown}}}{n_{\text{standard}}} \right)^2 \quad \text{Eq. 2}$$

where  $\Phi$  is the quantum yield,  $m$  is the slope of integrated fluorescence intensity against absorbance, and  $n$  is the refractive index of the solvent.

## 3. Results And Discussion

### 3.1. Structural Features of Metallafluorenes in this Study.

The available, previously reported metallafluorenes provide for an initial exploration of aqueous solution behavior (Fig. 1A). MFs with both Si (**1**, **3** and **4**) and Ge centers (**2**) are included; both mono (**3**, **4**) and bicyclic (**1**, **2**), fused (**2**) and not fused (**1**) 2,7 substituents are represented. Importantly for aqueous applications, there is variety in the polarity of this group among these compounds. Of particular interest is **2**, which features a 6-methoxy naphthyl substitution, as naphthyl groups are common in fluorophores that interact with surfactants [3, 4, 8, 7].

### 3.2. Group 14 Metallafluorenes are Soluble and Luminescent in Aqueous Solution.

Stock solutions of compounds **1–4** were prepared in DMSO as determined by mass. Extinction coefficients were calculated from absorbance spectra and confirmed from the slope of a line fit to

absorbance data as a function of concentration. See Table 1. These extinction coefficients were then used to prepare solutions for the remainder of the study. Despite significant hydrophobic character, if dispensed from a DMSO stock near 1 mM, these MFs are water soluble in the low micromolar range, sufficient for spectroscopic work.

Table 1  
Optical Properties of Metallafluorenes 1–4 in Aqueous Solution.

Compd	$\epsilon^a$ $M^{-1}cm^{-1}$	Abs $\lambda_{max}^b$ nm	Em $\lambda_{max}^b$ nm	Stokes Shift nm
<b>1</b>	28,300	387	453	66
<b>2</b>	73,400	390	483	87
<b>3</b>	59,700	376	465	89
<b>4</b>	56,400	376	464	88

<sup>a</sup>In DMSO at Abs  $\lambda_{max}$ . <sup>b</sup>Conditions: 10 mM Tris, 5% DMSO, pH 8.0, 25°C

From DMSO stocks, aqueous solutions were prepared in 10 mM Tris buffer, pH 8.0 (a common biological buffer) with 5% or less of DMSO and both absorbance and emission spectra obtained. It is clear from Fig. 2 that the 2,7 substituent provides an optical tunability of spectra in aqueous solution of up to 14 nm (376–390 nm) at absorption. The same is true of emission, but here the range is 30 nm. **1** is the most blue shifted of the group, while **2** is the most red shifted. Indeed, using <https://www.molinspiration.com/cgi-bin/properties> to compute logP for the 2,7 substituents confirms that the biphenyl substituent is the most lipophilic (hydrophobic) with a logP of 4.31 and the tolyl and phenylfluorine substituents are the least lipophilic (2.96 and 2.65, respectively). While the methoxynaphthyl substituent has a high logP value (3.73), it is the only one of this series with a Ge center. Stokes shifts range from 66 to 89 nm. Spectroscopic parameters are summarized in Table 1.

### 3.3. Metallafluorene Spectroscopic Behavior in the Presence of Surfactants.

#### 3.3.1. Absorption Behavior

The hydrophobicity of the MFs suggests that they could interact with detergents/surfactants. To explore this, absorption spectra were collected for **1–4** in the presence of 5 surfactants that vary with respect to charge at a concentration above their respective CMC values (TX, CHAPS, CTAB, SDS, and SDBS; see **Table S1** for a summary of properties). In all cases, the spectrum of the surfactant itself is subtracted from that of the mixture (Fig. 3). TX elicited the largest enhancements in absorbance, and in the case of **2** and **3**, the absorbance approached that obtained in DMSO. The spectral absorbance changes are the

most interesting with **2** and **4**. For these MFs, changes in peak shape are also observed. CTAB introduces scatter for **4** (see intensity between 425 and 450 nm) relative to CTAB itself, the contribution of which has been subtracted. This suggests a change in particle size and thus a direct interaction that affects both the MF and the surfactant (see Sect. 3.5 for more detail). In contrast, the spectral responses to the anionic surfactants SDS and SDBS were very small to insignificant for all four MFs. This is typical among fluorescent surfactant indicators [3–5, 9].

### 3.3.2. Emission Behavior.

Figure 4 visually summarizes the effects of various surfactants (above their CMC) on the emission behavior of **2**. Using emission in buffer and DMSO as references, it is clear that for this MF, CTAB and TX induce the greatest emission enhancements upon excitation at 350 nm, while the anionic detergents show little enhancement.

This is reflected in the spectral data summarized in Fig. 5, Fig. 6, and Table 2 for all four compounds. In general, the spectral responses to TX are the most dramatic: a large blue shift is observed in all cases (37–63 nm of  $\lambda_{\max}$ ), which is consistent with an interaction between the MF and the surfactant that reduces exposure to aqueous solvent and its relaxation effects. In addition, probably due to the same effect, the electronic transitions are quite distinct in the spectra. Further, the fold emission enhancements are large (6–19 fold). Given that TX is a neutral and aromatic surfactant, this seems reasonable and is comparable to that observed with other indicators [8, 14].

Similar effects are also observed for **2** and **3** towards CHAPS and CTAB, and fold enhancements are similar to that for TX. A standout is **1**, which shows a 25-fold enhancement in the presence of CTAB. In contrast,  $\lambda_{\max}$  shifts and emission enhancements are small or negligible upon the addition of anionic surfactants SDS and SDBS to all compounds. This is consistent with the literature [4, 6, 9, 10].

Table 2  
Effects of Surfactants on Metallafluorene Emission<sup>a</sup>

Comp	TX		CTAB		CHAPS		SDS		SDBS	
#	$\lambda^b$	FE <sup>c</sup>								
1	38	19 ± 0	37	25 ± 10	37	2.0 ± 0.6	2	1.1 ± 0.1	2	0.9 ± 0.02
2	63	13 ± 2	63	12 ± 0	63	1.7 ± 0.04	1	1.0 ± 0.2	0	1 ± 0.1
3	58	13 ± 5	58	9 ± 3	62	3.3 ± 0.4	0	0.9 ± 0.2	3	1 ± 0.4
4	60	10 ± 1	60	5 ± 2	2	4.0 ± 0.2	1	1.1 ± 0.2	1	1.1 ± 0.3

<sup>a</sup>Conditions: 1  $\mu$ M MF, 10 mM Tris pH 8.0; 0.5 % DMSO, 25°C. TX, 0.6 mM; CHAPS, 25 mM; CTAB, 20 mM; SDS, 30 mM; SDBS, 10 mM. All of these surfactant concentrations are above their respective CMC. <sup>b</sup>Lambda refers to shift in nm of  $\lambda_{max}$  upon the addition of surfactant. <sup>c</sup>Fold enhancement is calculated by dividing the maximum emission with surfactant by the maximum emission in the absence of surfactant with a 3 min incubation

### 3.3.3. Effect of Surfactants on Metallafluorene Quantum Yields.

Quantum yields ( $\Phi$ ) were measured using coumarin 102 in ethanol as a standard as described in Sect. 2.3.3. As summarized in Table 3,  $\Phi$  values in Tris buffer range from 0.09 to 0.34. On the low end are **2** and **4**.  $\Phi$  values for these in water fall in a similar range with common probes 8-Anilinonaphthalene-1-sulfonic acid (ANS; 0.004; [15]), tryptophan (0.13; [16]), and coumarin (0.09; [17]), as well as with DMNDC (0.01; [8]). However, for **3** and **4**,  $\Phi$  in water is relatively high (near 0.3). Common xanthene dyes like Rhodamine B and Texas Red have quantum yields of 0.31 and 0.35 in water [18], which is considered respectable for biological probes. **4** has a comparable quantum yield to this family of dyes and a greater quantum yield than dyes like Cy3 and Cy5 (quantum yields 0.04 and 0.27, respectively) [19] and the surfactant indicator N-n-octyl-4-(1-methylpiperazine)-1,8-naphthalimide iodide (0.24; [3]).

$\Phi$  values are at almost 100% quantum efficiency in the presence of TX and CTAB, indeed higher than in the organic solvent DCM, and  $\Phi$  values for all but **2** are above 0.8 in CHAPS. The  $\Phi$  values in the presence of anionic surfactants are much lower (0.38–0.55), but all of these are higher than any observed in buffer alone.

Table 3  
Effect of Surfactants on Metallfluorene Quantum Yield  $\phi^a$

Compd	10 mM Tris pH 8	TX	CTAB	CHAPS	SDS	SDBS	DCM <sup>b</sup>
1	0.11 ± 0.016	0.94 ± 0.05	0.92 ± 0.08	0.90 ± 0.02	0.49 ± 0.09	0.38 ± 0.13	0.80
2	0.094 ± 0.011	0.85 ± 0.06	0.97 ± 0.03	0.52 ± 0.01	0.38 ± 0.06	0.46 ± 0.006	0.75
3	0.24 ± 0.013	0.97 ± 0.04	1.01 ± 0.11	0.80 ± 0.08	0.53 ± 0.03	0.55 ± 0.01	0.89
4	0.34 ± 0.005	1.00 ± 0.04	0.92 ± 0.08	0.90 ± 0.02	0.49 ± 0.09	0.38 ± 0.13	0.80

<sup>a</sup>Conditions: 1  $\mu$ M MF, 0.6 mM TX, 20 mM CTAB, 25 mM CHAPS, 30 mM SDS, 10 mM SDBS in 10 mM Tris, pH 8.0, 5% DMSO, 25°C. <sup>b</sup>From Hammerstroem et al. [1, 2].

### 3.3.4. Effect of Ions on Metallfluorene Emission Behavior.

To address specificity of emission enhancements induced by surfactants, the effects of various ions on MF emission were examined. As summarized in Fig. 7, generally very small reductions in emission were observed. And while they are the same scale as the enhancements observed in the presence of anionic surfactants, they are dwarfed by the changes upon the addition of TX, CTAB, and CHAPS. Thus the response to ions is very small and in an opposite direction to those observed for surfactants, indicating a high degree of specificity toward surfactants.

### 3.5. Probing Metallfluorene-Surfactant Interactions.

The above data suggest an interaction between MFs and surfactant micelles. To probe this possibility, MF anisotropies were collected in the presence and absence of surfactant (Fig. 8). When MFs are added to TX, CHAPS, and CTAB, there is an obvious large increase in anisotropy. This is consistent with association of MF with the surfactant micelle. Coupled with spectral changes (blue shifts), fold enhancements and quantum yields, these data are consistent with substantial protection of MFs from polar solvent and its relaxation effects. This is in sharp contrast to the effect of anionic surfactants, which generally result in lower fold-enhancements, quantum yields, and anisotropies. This latter pattern is consistent with either weak interactions with aggregates or an association with smaller surfactant structures that provide little to no protection from solvent relaxation and no evidence that MFs are highly sequestered in hydrophobic environments.

To better understand the interaction of MF with a surfactant micellar surface, TX was titrated into **4**. As shown in Fig. 9, emission increases dramatically at 200  $\mu$ M, commonly reported as the CMC of TX [8, 20].

This is also consistent with a sensitivity toward aggregates surfaces and thus a direct interaction with them.

## 4. Conclusions

The results here demonstrate the potential of MFs as probes of surfactants. First, substitution at the 2,7 position offers an opportunity to tune desirable properties that could include water solubility as well as electronic properties that could include  $I_{\max}$  and quantum yield. Second, they have sufficient water solubility and favorable luminescence behaviors for use in aqueous solution. Third, the differences in MF responses to various surfactants suggest sensitivities to surfactant charge, aggregate size, and CMC [3, 5, 7], any of which could make them useful probes of these properties. **1** exhibits the highest fold enhancements toward neutral and cationic surfactants; **2** exhibits the most distinct spectral responses to the various surfactants. Both have bicyclic 2,7 substituents and suggest a pathway for the development of second-generation MF probes.

We hypothesize that these compounds are intramolecular charge transfer (ICT) dyes that contain an electron rich moiety and electron deficient moiety connected by conjugated bonds. As an ICT dye, they may have future applications as aggregation-induced emission enhancing probes for protein, nucleic acid, and polysaccharide detection [21]. Given their sensitivity to surfactant aggregates, MFs would be especially useful probes of the properties of lipids and biological membranes. This could include detecting changes in local membrane lipid composition in response to a molecular or cellular stimulus [22] as well as imaging cellular structures that have differing lipid compositions [22]. There are also potential applications in probing protein behavior through the detection of conformational changes (that often involve changes in accessible nonpolar surface area) [23]. Finally, through conjugation with a biomolecule of interest, MFs could be used to measure proximity or distance via FRET with chromophores located on biomolecular binding partners (e.g., lipids, proteins, and nucleic acids) [24]. Investigations into the electronic mechanisms of these MFs and their potential applications are ongoing.

## 5. Declarations

**Author Contributions.** All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Helena Spikes, Shelby Jarrett-Noland and Stephan Germann. The first draft of the manuscript was written by Cynthia Dupureur and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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**Conflict of Interest.** The authors have no conflicts of interest to declare that are relevant to the content of this article.

**Data Availability.** The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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## Figures

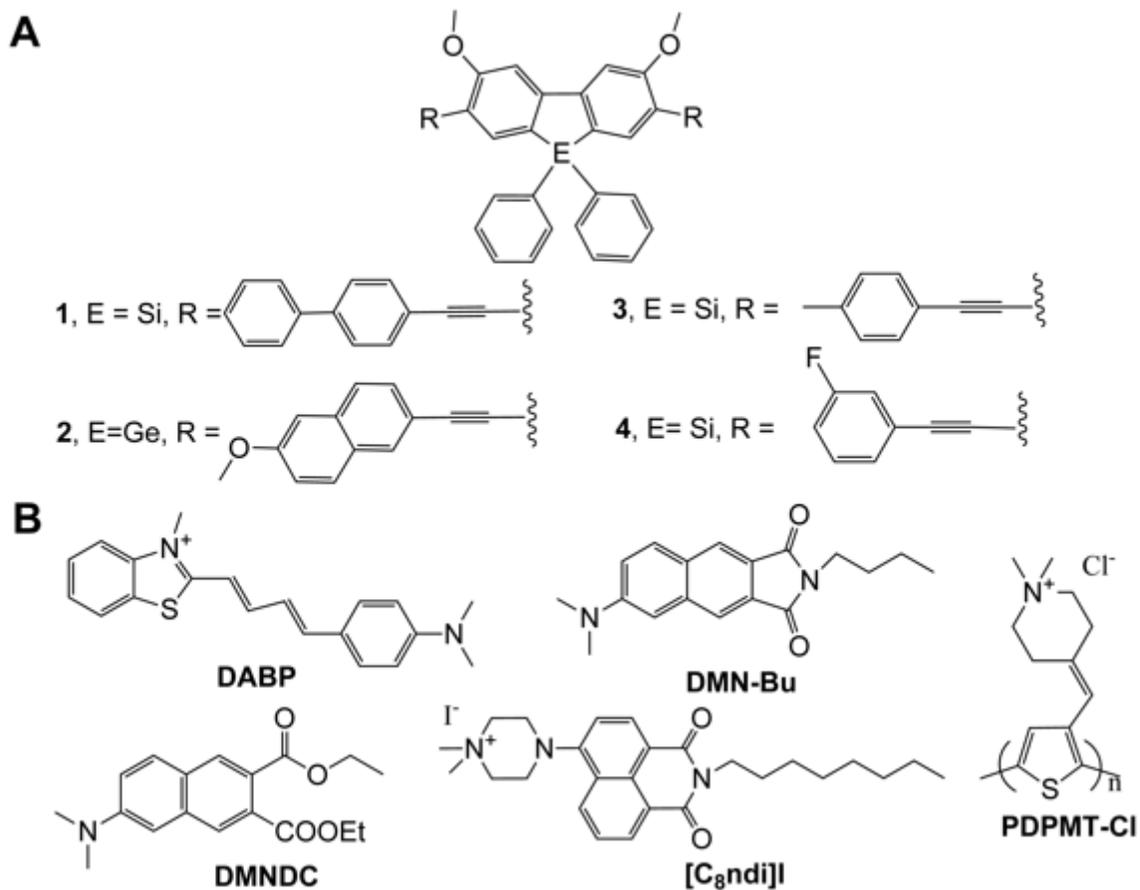


Figure 1

A: Structures of metallafluorenes in this study. B: Examples of fluorescent surfactant indicators [8,7,9,3]

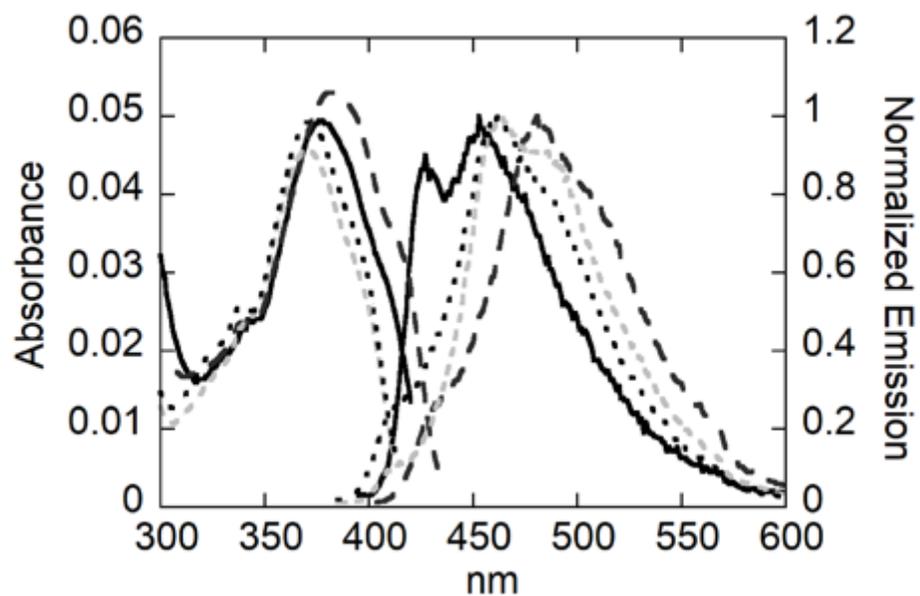
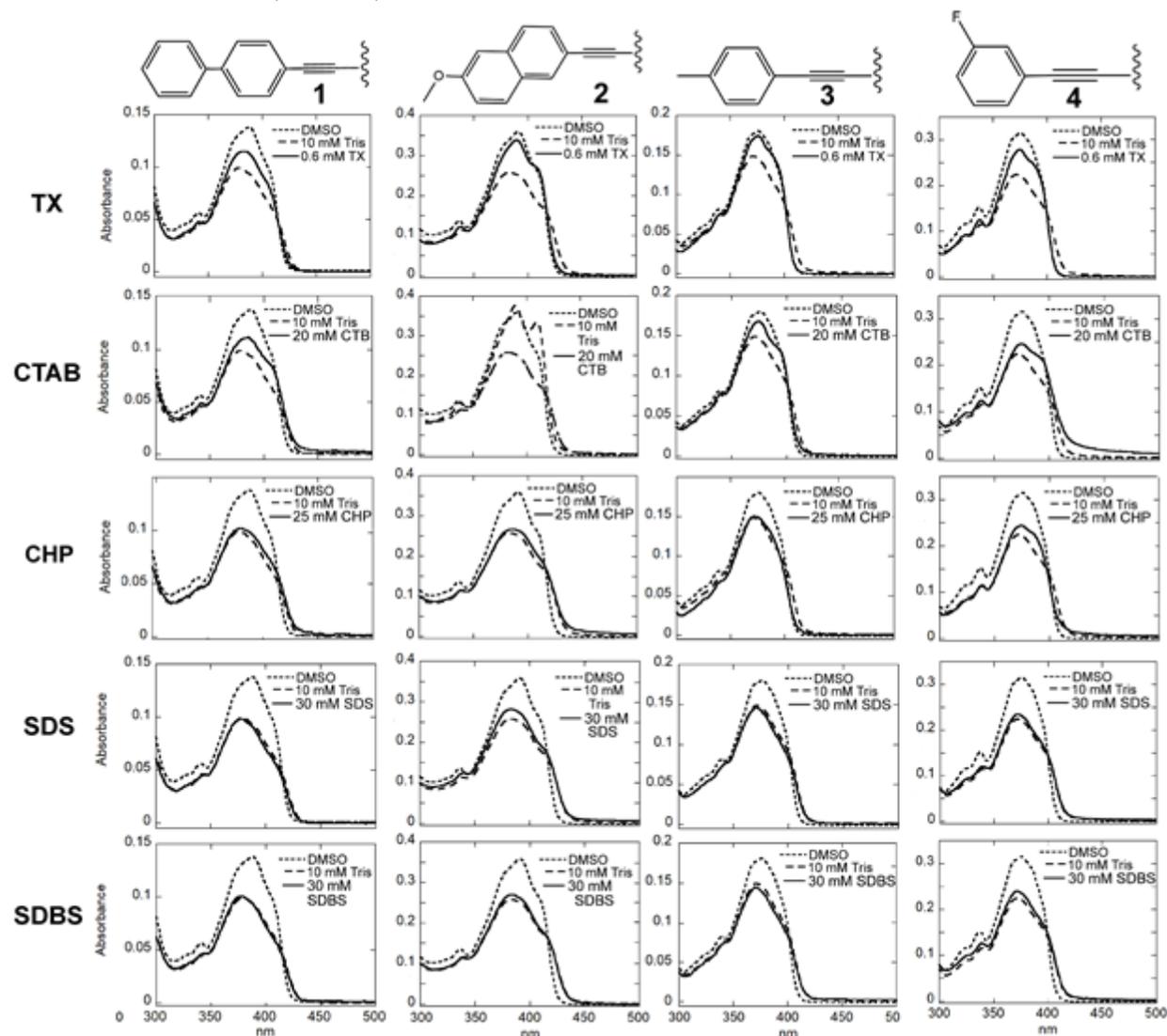


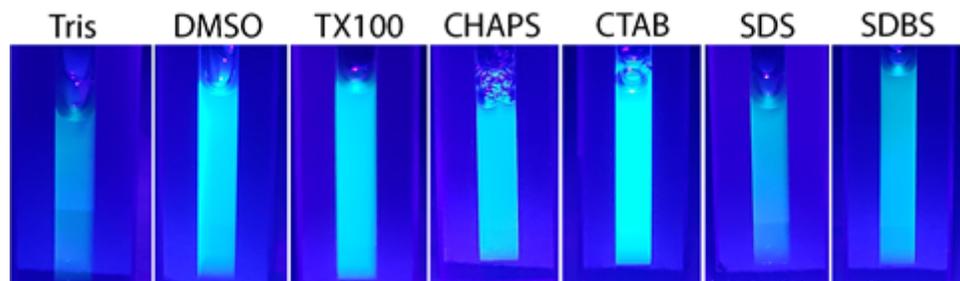
Figure 2

Absorption (solid) and emission (dashed) spectra of 1-4 in aqueous solution. Conditions: 10 mM Tris, pH 8.0, 0.5-0.8% DMSO, 25 °C. All MF concentration are 1  $\mu$ M, but due to lower  $\epsilon$ , the absorbance of 1 is adjusted mathematically for easy comparison. Excitation wavelengths were at the absorption maximum with 1 nm slitwidth (Table 1)



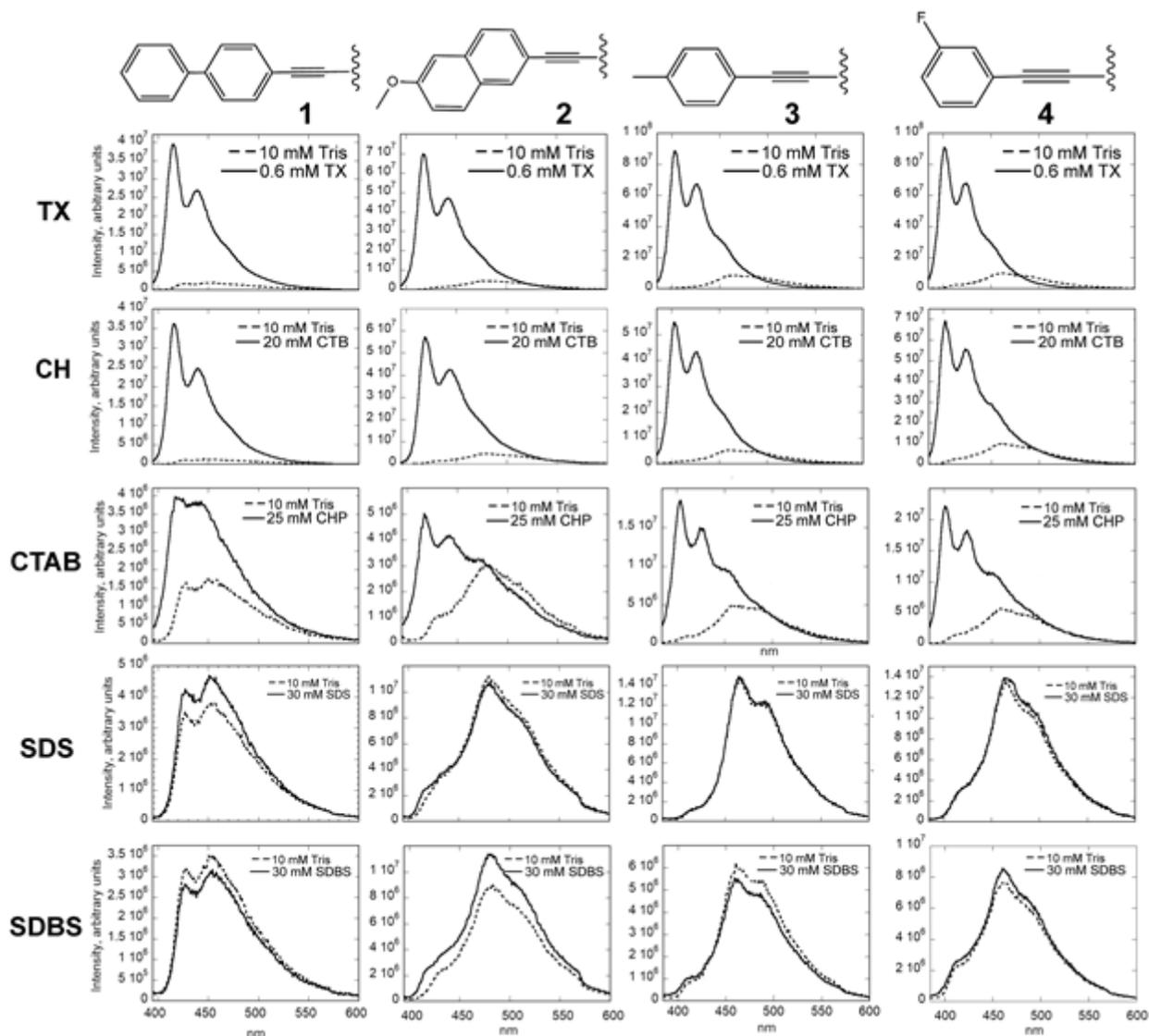
**Figure 3**

Absorption spectral behavior of 1-4 in aqueous solution upon the addition of surfactants. Conditions: 5  $\mu$ M MF, 10 mM Tris, pH 8.0, 0.5% DMSO, 25 °C, 0.6 mM TX, 25 mM CHAPS, 20 mM CTAB, 30 mM SDS, and 10 mM SDBS



**Figure 4**

Visible luminescence upon excitation at 350 nm of 5  $\mu\text{M}$  of **2** in 10 mM Tris buffer pH 8 and 0.5% DMSO alone and also in the presence of various surfactants at room temperature: TX, 0.6 mM; CHAPS: 25 mM; CTAB, 20 mM; SDS, 30 mM; SDBS, 10 mM. All of these surfactant concentrations are above their respective CMC values



**Figure 5**

Effect of Various Surfactants on Metallfluorene Emission Spectra. 1  $\mu\text{M}$  MF in 10 mM Tris, 0.5% DMSO, pH 8.0, 25  $^{\circ}\text{C}$ , with as indicated 0.6 mM TX, 25 mM CHAPS, 20 mM CTAB, 30 mM SDS, 10 mM SDBS. All of these surfactant concentrations are above their respective CMC value

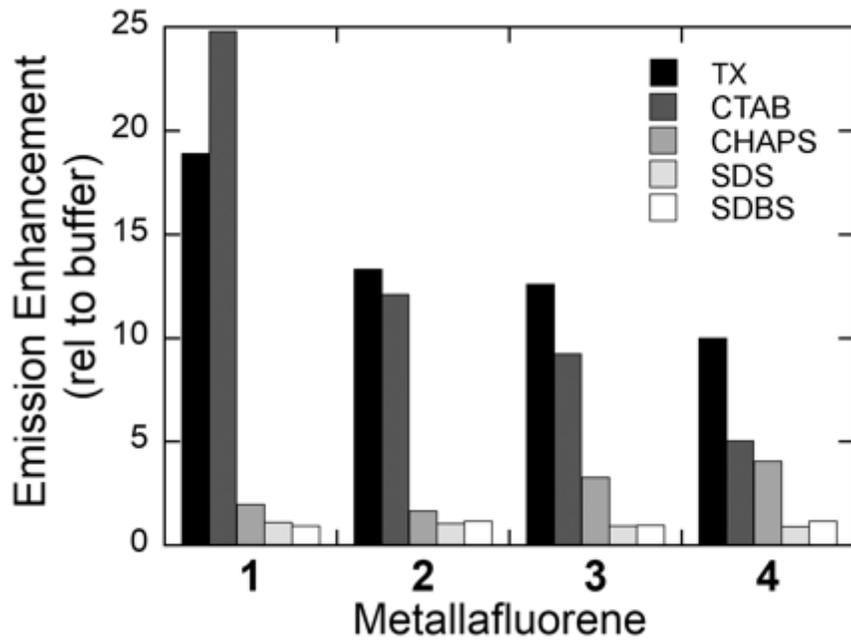


Figure 6

Summary of Emission Metallafuorene Enhancements Induced by Surfactants. 1  $\mu\text{M}$  MF, 10 mM Tris, 0.5 % DMSO, pH 8.0, 25  $^{\circ}\text{C}$ . Conditions as in Table 2

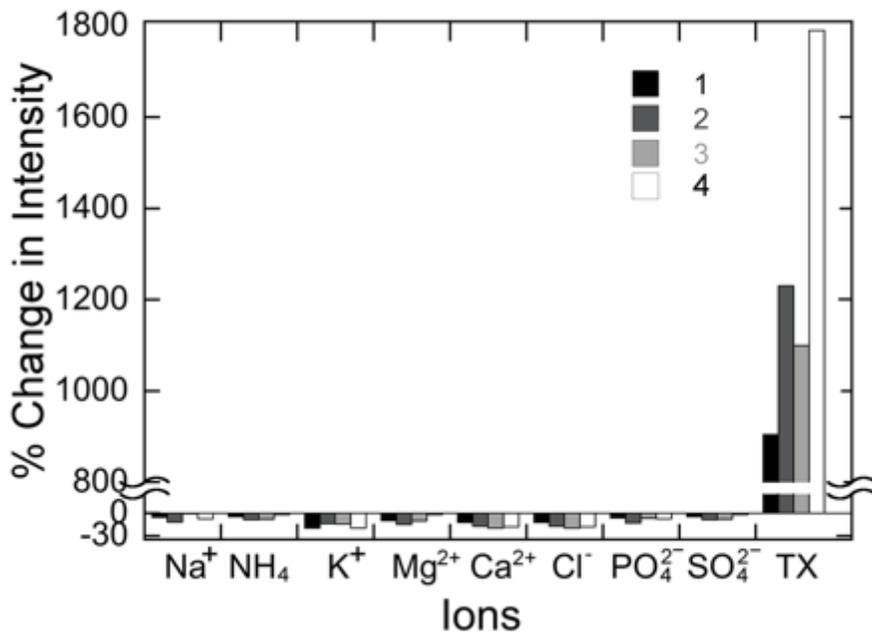


Figure 7

Effects of Ions on Metallafuorene Fluorescence. Conditions: 1  $\mu\text{M}$  MF, 1 mM salt, 10 mM Tris, pH 8.0, 0.5 % DMSO, 25  $^{\circ}\text{C}$ . TX concentration is 0.6 mM

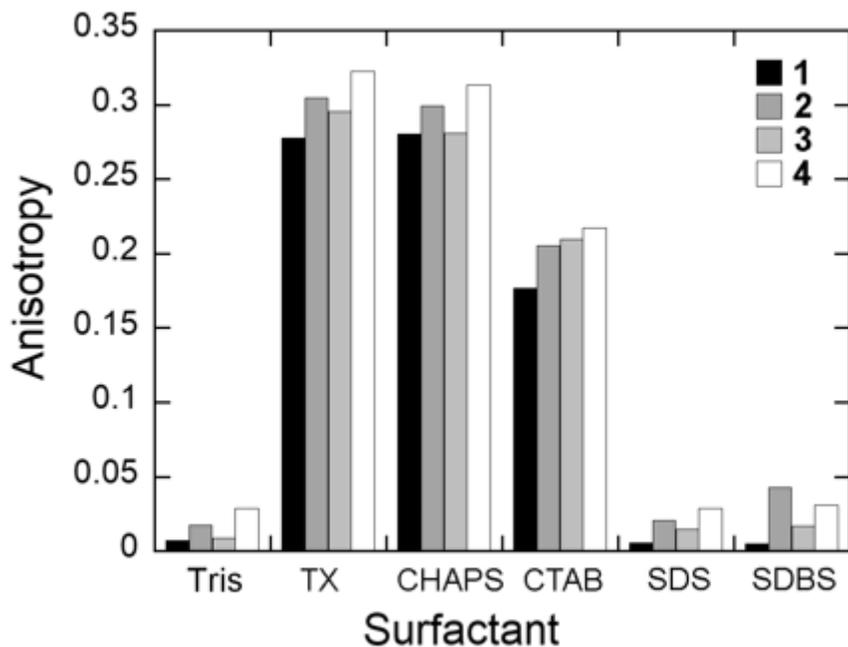


Figure 8

Anisotropies of Metallafuorenes in the Presence of Surfactants. Conditions: 1  $\mu\text{M}$  MF, 10 mM Tris, pH 8.0, 0.5% DMSO, 25  $^{\circ}\text{C}$ . 0.6 mM TX, 20 mM CTAB, 25 mM CHAPS, 30 mM SDS, 10 mM SDBS. Anisotropies were collected in triplicate with an error of no more than 10%

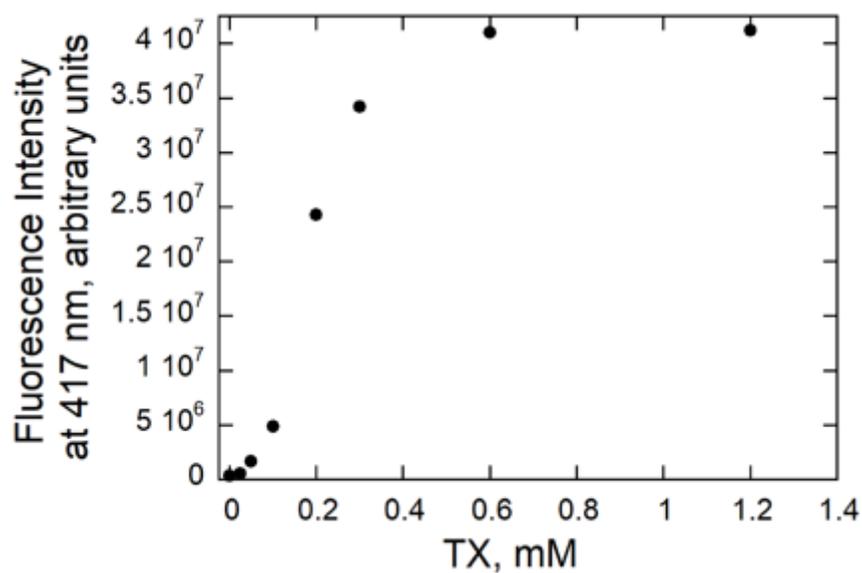


Figure 9

Equilibrium Titration of TX into MF. Conditions: 1  $\mu\text{M}$  4, 10 mM Tris, pH 8.0, 0.5% DMSO, 25  $^{\circ}\text{C}$

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [TableS1DetergentPropertiesHS91017.docx](#)