Supporting Information for:

New Pyrenyl Fluorescent Amphiphiles: Synthesis and Aggregation Properties

Cecilia Bombelli[a], Federico Bordi[b,c], Stefano Borocci[d*], Marco Diociaiuti[e], Raffaella Lettieri[a], Francesca Limongelli[a], Giovanna Mancini[a], Simona Sennato[b,c]*

a CNR, Istituto di Metodologie Chimiche and Università degli Studi di Roma "La Sapienza", P.le A. Moro 5, 00185 Roma Italy, b Dipartimento di Fisica, Università degli Studi di Roma "La Sapienza", P.le A. Moro 5, 00185 Roma Italy, c CNR-IPCF, Università di Roma "La Sapienza," P.le A. Moro 5, 00185 Roma, Italy, d Dipartimento di Scienze Ambientali, Università della Tuscia, Largo dell'Universita' 01100 Viterbo, e Dipartimento di Tecnologie e Salute Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Roma Italy

Synthesis of surfactants 1-4

*1-(1-pyrenyl)-11-bromo-1-undecanone(**I**).*
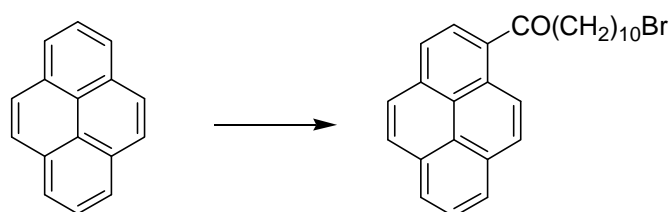
In a 5 mL round bottom flask, 1.8 mL (24.8 mmol) of thionyl chloride were added to 1.0 g (3.8 mmol) of 11-bromoundecanoic acid. The mixture was heated at 40°C for 4 h and then stirred at room temperature for 12 h. After removal of the excess of thionyl chloride by distillation, the residual colourless oil was dissolved in 6 mL of dry CH_2Cl_2, transferred in a two neck round bottom flask, and cooled to 0°C in a ice bath. Upon addition of 0.72 g (3.6 mmol) of pyrene and 0.57 g (4.3 mmol) of $AlCl_3$, the reaction was stirred for 5 h at 0°C. The reaction mixture was then poured on an ice/water/Et_2O mixture. The organic layer was washed three times with water and dried over Mg_2SO_4. After removal of solvent, purification of the crude product on silica gel, using hexane/Et_2O (9:1) as eluent, yielded the intermediate product **I** as a yellow solid (98%). ^1H NMR, δ ($CDCl_3$): 1.2-1.5 (m, 14H, aliphatic chain); 1.9 (m, 2H, CH_2 ω-1); 3.2 (t, 2H, CH_2 α); 3.4 (t, 2H, CH_2 ω); 7.9-8.3 (m, 8H, Ar); 8.9 (d, 1H, Ar).
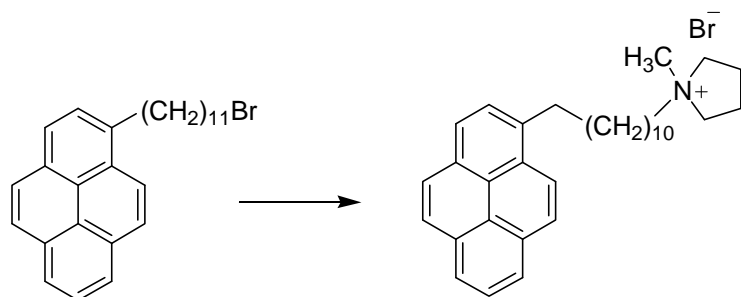
*11-(1-pyrenyl)-1-bromoundecene(**II**).*
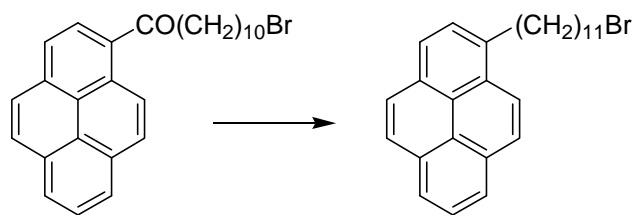
11-(1-pyrenyl)-1-bromoundecene was prepared according to a reported procedure[1]. Briefly, in a two neck round bottom flask fitted with a reflux condenser, 0.45 g (3.4 mmol) of $AlCl_3$ dissolved in 5 mL of dry Et_2O were added dropwise to a 1 M solution of $LiAlH_4$ in dry Et_2O. Then, 0.5 g (1.1 mmol) of **I** dissolved in 10 mL of dry CH_2Cl_2 were added dropwise to the mixture cooled to 0 °C in a ice bath, and the reaction mixture was stirred at room temperature and monitored by TLC (hexane/Et_2O 9:1) until disappearance of **I** (~3 h). The reaction was quenched by the addition of 10 mL of Et_2O and 10 mL of water; the organic layer was washed several times with HCl 1 M and dried over Mg_2SO_4. Removal of the solvent yielded a yellow solid (90%). ^{1}H NMR, δ ($CDCl_3$): 1.0-1.5 (m, 16H, aliphatic chain); 1.8 (m, 4H, CH_2 α, CH_2 ω-1); 3.3 (m, 4H, CH_2 ω, CH_2 β); 7.7-8.2 (m, 9H, Ar).

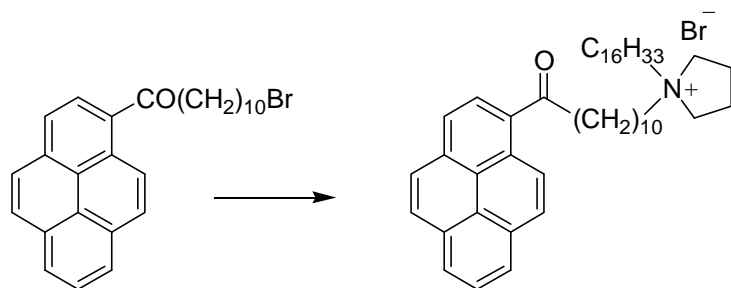*N-methyl-N-[11-(1-pyrenyl)-11-oxoundecyl]pyrrolidinium bromide (**1**).*
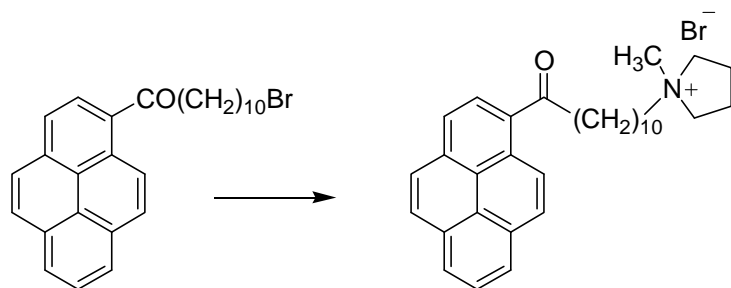
0.13 mL (1.2 mmol) of N-methylpyrrolidine dissolved in 10 mL of Et_2O/CH_3OH (1:1) were added to 0.5 g (1.1 mmol) of **I**. The reaction mixture was kept under stirring at 40°C for 15 d and monitored by TLC (hexane/Et_2O 9:1). After removal of the solvent, the crude product was washed with hexane, and crystallized from acetone. The target compound **1** was obtained as a yellow solid (51%). mp = 114 °C (±2 °C). Elemental analysis for $C_{32}H_{40}BrNO$: calcd C 71.90%; H 7.54%; Br 14.95%; N 2.62%; O 2.99%. found: C 71.50%; H 7.80%, N 2.53%. ^1H NMR, δ ($CDCl_3$): 1.3 (m, 12H, aliphatic chain); 1.6 (m, 2H, CH_2 ω-1); 1.8 (q, 2H, CH_2 β); 2.2 (m, 4H, CH_2 3, 4); 3.1 (s, 3H, N-CH_3); 3.2 (m, 2H, CH_2 α); 3.6 (m, 2H, CH_2 ω); 3.7 (m, 4H, CH_2 2, 5); 8.0-8.3 (m, 8H, Ar); 8.9 (d, 1H, Ar). ^{13}C NMR, δ ($CDCl_3$): 1.04, 14.15, 21.62, 22.67, 24.00, 24.89, 26.31, 29.09, 29.18, 29.26, 29.32, 31.61, 42.62, 48.57, 64.18, 76.69, 77.12, 77.54, 124.14, 124.75, 124.96, 125.97, 126.05, 126.26, 126.46, 127.14, 129.13, 129.41, 129.46, 130.50, 131.08, 132.92, 133.55, 205.57.

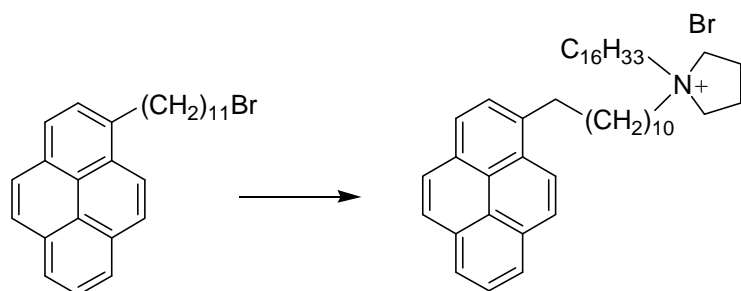*N-methyl-N-[11-(1-pyrenyl)-undecyl]pyrrolidinium bromide (**2**)*

N-methyl-N-[11-(1-pyrenyl)-undecyl]pyrrolidinium bromide was prepared by the quaternization reaction of N-methyl pyrrolidine with 11-(1-pyrenyl)-1-bromoundecene, **II**, according to the procedure reported for **1**. After removal of the solvent, the crude solid was washed with hexane to obtain the target compound **2** as a yellow solid (50%). mp = 116 °C(±2°C). Elemental analysis for $C_{32}H_{42}BrN$: calcd C 73.83%; H 8.13%; Br 15.35%; N 2.69%; found: C 73.50%; H 8.50%, N 2.67%. ^1H NMR, δ (CDCl$_3$): 1.2-1.5 (m, 14H, aliphatic chain); 1.5 (m, 2H, CH$_2$ ω-1); 1.8 (q, 2H, CH$_2$ β); 2.1 (m, 4H, CH$_2$ 3, 4); 3.2 (t, 2H, CH$_2$ α); 3.4 (m, 2H, CH$_2$ ω); 3.6 (m, 4H, CH$_2$ 2, 5); 7.8-8.3 (8d, t, 9H, Ar). ^{13}C NMR, δ (CDCl$_3$): 21.57, 24.02, 26.35, 29.17, 29.33, 29.41, 29.50, 29.54, 29.78, 31.97, 33.63, 48.46, 64.15, 64.34, 76.69, 77.12, 77.54, 123.60, 124.68, 124.82, 124.85, 125.02, 125.86, 126.50, 127.13, 127.36, 127.60, 128.57, 129.64, 130.92, 131.42.

N-hexadecyl-N-[11-(1-pyrenyl)-11-oxoundecyl]pyrrolidinium bromide (3).

N-hexadecyl-N-[11-(1-pyrenyl)-11-oxoundecyl]pyrrolidinium bromide was prepared by the quaternization reaction of N-hexadecyl pyrrolidine with 1-(1-pyrenyl)-11-bromo-1-undecanone, **I**, according to the procedure reported for **1**. After removal of the solvent, the crude product was washed with hexane, and crystallized from ethyl acetate. The target compound **3** was obtained as a yellow pale solid (50%). mp = 83°C (±2°C). Elemental analysis for $C_{47}H_{70}BrNO$: calcd C 75.78%; H 9.47%; Br 10.73%; N 1.88%; O 2.15%. found: C 75.38%; H 9.84%, N 1.86%. ^1H NMR, δ (CDCl$_3$): 0.9 (t, 3H, CH$_3$); 1.3 (m, 38H, aliphatic chains); 1.6 (m, 2H, CH$_2$ β'); 1.7 (m, 2H, CH$_2$ β); 1.8 (q, 2H, CH$_2$ ω-1); 2.2 (m, 4H, CH$_2$ 3, 4); 3.1 (t, 2H, CH$_2$ ω); 3.2 (m, 4H, CH$_2$ α, α'); 3.7 (m, 4H, CH$_2$ 2, 5); 8-8.3 (7d, t, 8H, Ar); 8.8 (d, 1H, Ar). ^{13}C NMR, δ (CDCl$_3$): 1.056, 14.17, 21.76, 22.73, 23.50, 24.90, 26.35, 26,40, 29.09, 29.19, 29.27, 29.32, 29.40, 29.50, 29.63, 29.71, 31.96, 42.63, 59.29, 62.88, 76.65, 77.08, 77.50, 124.12, 124.38, 124.79, 125.04, 126.01, 126.07, 126.29, 126.47, 127.17, 127.46, 129.23, 129.47, 129.51, 130.57, 131.13, 132.93, 133.63.

N-hexadecyl-N-[11-(1-pyrenyl)-undecyl]pyrrolidinium bromide (4).

N-hexadecyl-N-[11-(1-pyrenyl)-undecyl]pyrrolidinium bromide was prepared by the quaternization reaction of N-hexadecyl pyrrolidine with 11-(1-pyrenyl)-1-bromoundecene, **II**, according to the procedure reported for **1**. After removal of the solvent, the crude product was washed with hexane, and crystallized from ethyl acetate. The target compound **4** was obtained as a yellow pale solid (60%). mp = 57°C (±2°C). Elemental analysis for $C_{47}H_{72}BrN$: calcd C 77.22%; H 9.93%; Br 10.93%; N 1.92%. found: C 76.94%; H 10.33%, N 1.94%. ^1H NMR, δ (CDCl$_3$): 0.9 (t, 3H, CH$_3$); 1.4 (m, 38H, aliphatic chains); 1.6 (q, 2H, CH$_2$ ω-2); 1.7 (m, 4H, CH$_2$ β,β'); 1.9 (q, 2H, CH$_2$ ω-1); 2.2 (m, 4H, CH$_2$ 3, 4); 3.3 (m, 6H, CH$_2$ α, α', CH$_2$ ω); 3.8 (m, 4H, CH$_2$ 2, 5); 7.8-8.3 (8d, t, 9H, Ar). ^{13}C NMR, δ (CDCl$_3$): 14.17, 21.80, 22.72, 23.47, 26.36, 29.14, 29.19, 29.33, 29.40, 29.51, 29.65, 29.69, 31.95, 33.61, 59.34, 62.92, 123.56, 124.66, 124.82, 125.05, 125.83, 126.50, 127.12, 127.32, 127.58, 128.59, 129.67, 130.93, 131.44, 137.36.

Conductometric measurements

Krafft temperatures of surfactants **1-2** were determined by conductivity measurements according to a described procedure[2,3]. Weighted quantities of surfactant **1-2** were added to water and the mixtures were heated to obtain clear solutions. The aqueous solutions were kept at 277 K for 24 h before measurement. During the measurements, the solutions were continuously stirred and the temperature was raised at a rate of 0.1 K/min.

Determination of the critical aggregation concentrations, cacs, of **1-4** was carried out by conductivity measurements[4]. Experiments were carried out at 328 K (above the Krafft temperature) by adding known volumes of a surfactant stock solution in bidistilled water (**1**, **2**) or absolute EtOH (**3**, **4**) to a fixed volume of deionized water and reading the conductivity values after equilibration at each addition. When a EtOH surfactant stock solution was used, the organic solvent added was ≤2% of the total volume, thus with a negligible effect on the surfactant aggregates and their aggregation parameters[5].

The conductivity values were plotted versus the concentration of surfactant and cacs were obtained from a nonlinear curve fitting the data to Equation (1)[6,7] using the Marquardt-Levenberg method,

$$\kappa = \kappa_0 + A_1 c + d(A_2 - A_1)\ln\left(\frac{1 + e^{(c-cac)/d}}{1 + e^{-cac/d}}\right) \qquad (1)$$

were κ and κ_0 are the specific conductivity of the solution and of the solvent respectively, c is the molar concentration of surfactant, cac is the critical aggregation concentration, A_1 and A_2 represent the slope of the premicellar and postmicellar region and d is a parameter that indicates if the aggregation occurs in a narrow or wide range of surfactant concentrations.
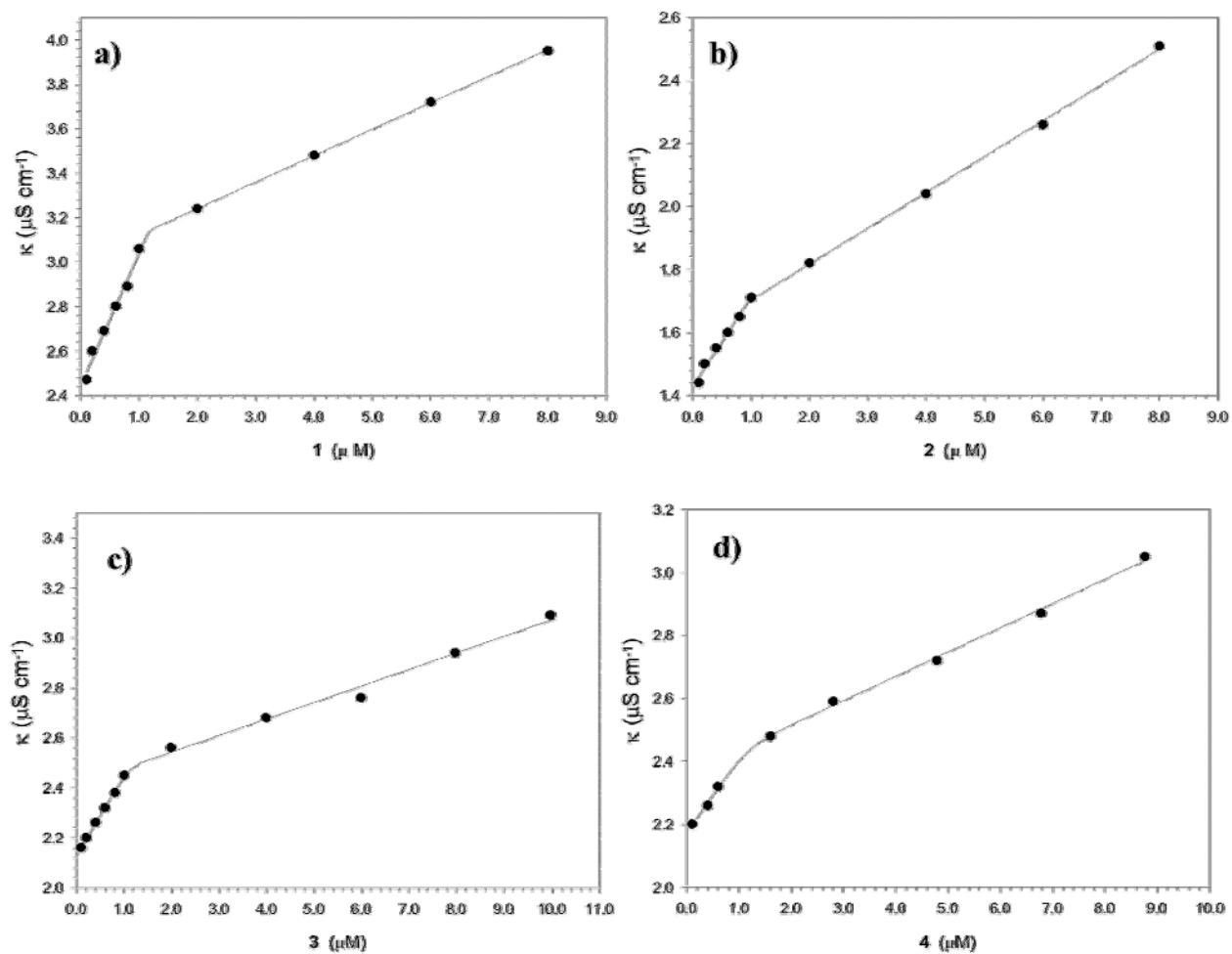
Figure S1. Specific conductivity vs concentration of **a) 1**, **b) 2** at 328 K and of **c) 3**, **d) 4** at 298 K

Spectroscopic measurements

Absorption spectra

Figure S2a shows the absorption spectra of surfactants **1-4** 10^{-5} M in absolute EtOH. The spectra of compounds **2** and **4** show the typical bands of alkylpyrene derivatives, i.e. all the electronic transitions higher than S1←S0 show vibrational fine structure. Absorption spectra of compounds **1** and **3** are, as expected for acyl-pyrene derivatives, red shifted, with the lowest energy absorption band at 353 nm, and only the S4←S0 band shows some vibrational fine structure. The spectral features of the aggregates formed by the amphiphiles in water (i.e. above the cac) are similar to those in EtOH, with the exception of a modest red shift of the bands of alkyl derivative **4** with respect to the bands of **2**. This result suggests that in aggregates of surfactant 4 the pyrene ring is surrounded by a more polar environment (Figure S2b).
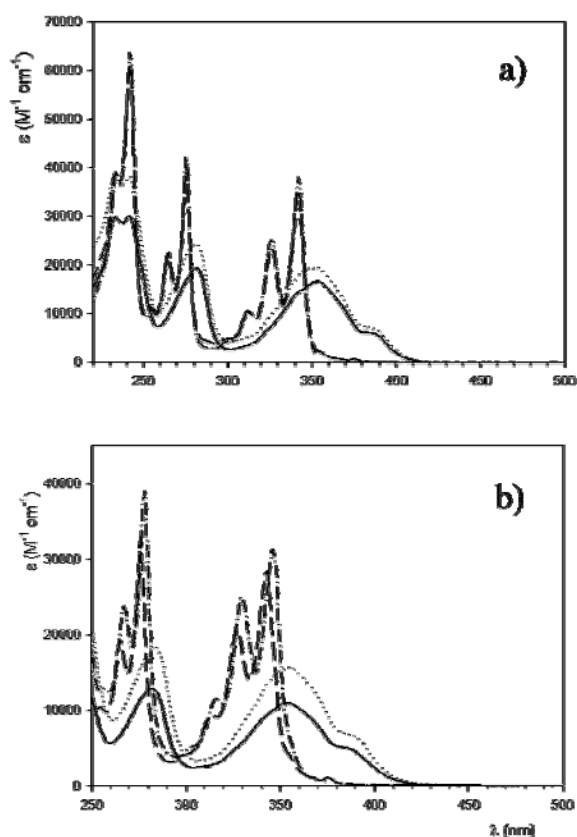

Figure S2. Normalized UV-visible spectra of surfactants **1**(solid), **2** (long dash), **3** (dotted), **4** (dash-dot) in ethanol a) and water b).

Fluorescence spectra of the monomers (in ethanol).

In Figure S3 we report the fluorescence spectra of surfactants **1-4** 10^{-5} M in absolute EtOH. The spectra of amphiphiles **2** and **4** (Figure S3b) display the characteristic fluorescence features of a pyrene monomeric moiety, i.e. in conditions in which the excimer does not form[8].On the other hand, the fluorescence spectrum of surfactant **1** (Figure S3a, solid line) is characterized by a broad, structureless band, with a maximum at 431 nm, typical of pyrene acyl derivatives in polar solvents with a dielectric costant ≥ 10. The fluorescence spectrum of compound **3** (Figure S3a, medium dash) shows three bands at 386, 407 and 430 nm, thus suggesting that the fluorophore experiences a hydrophobic and a polar environment, at the same time.
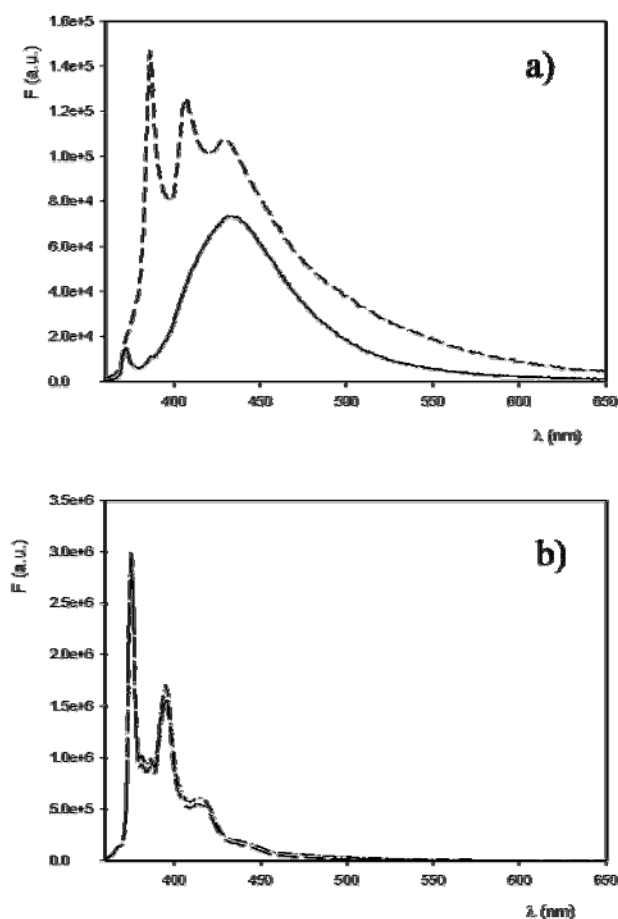

Figure S3. Emission fluorescence spectra of an ethanol solution 10^{-5} M of the different surfactants a) **1**(solid), **3** (medium dash), b) **2** (long dash), **4** (dash-dot).

The excitation spectra of compound **3** obtained at 390 nm (Figure S4, long dash) and 434 nm (Figure S4, dotted line), support this hypothesis. In fact the two spectra are qualitatively different, in particular, at the emission wavelength of 390 nm, all bands of the excitation spectrum are slightly more structured (similar to the absorption spectrum) and the band at the higher wavelengths is blue shifted.
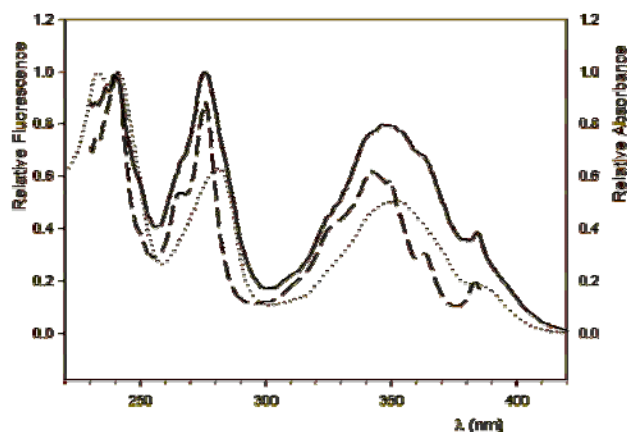

Figure S4. Excitation fluorescence spectra of an ethanol solution 10^{-5} M of surfactant **3** at λ_{em} 390 nm (long dash), and λ_{em} 430 nm (dotted), compared with the absorption spectrum (solid line).

Determination of quantum yields

The fluorescence quantum yields (Qf) of surfactants **1-4** were measured in EtOH. In order to obtain absolute quantum yields, anthracene was used as the fluorescence standard for **2** and **4**, and quinine sulphate in H2SO$_4$ 0.1 M for **1** and **3**[9]. As expected, the Qf of surfactants **1** (Qf = 0.011) and **3** (Qf = 0.031) is lower than Qf of **2** (Qf = 0.065) and **4** (Qf = 0.131), due to the presence of the carbonyl group linked to the pyrene ring.

Determination of the critical aggregation concentrations, cacs

The determination of cacs of surfactants **1-4** by fluorescence measurements were carried out at 328 K as described in the experimental section. Here we report the fluorescence emission spectra at 328 K in function of the concentration of surfactant **3** and **4** (Figure S5a and S6a, respectively) and the normalized fluorescence emission spectra (Figure S5b and S6b). The spectra of the aqueous solutions of **4** at low concentration (up to 8×10^{-7} M) clearly show, besides the bands relative to the monomer and the excimer, a third band at 580 nm due to the presence of ground state dimers (see inset, Figure S6a). Therefore these data were processed by considering only the 440-560 nm range of the spectrum, in order to exclude from the total intensity the effect of both the dimer and the monomer of the fluorophore.
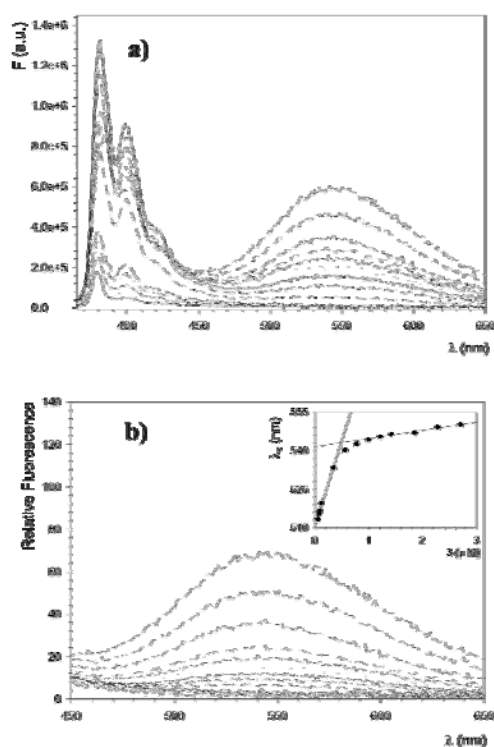

Figure S5. a) Emission fluorescence spectra at increasing concentrations (3×10^{-8}-3×10^{-6} M) of the surfactant **3**, all the spectra were recorded at 328 K; b) normalized emission fluorescence spectra at increasing concentrations (3×10^{-8}-3×10^{-6} M) of surfactant **3**; inset: the weighted wavelength λ_w versus the concentration of surfactant **3**.
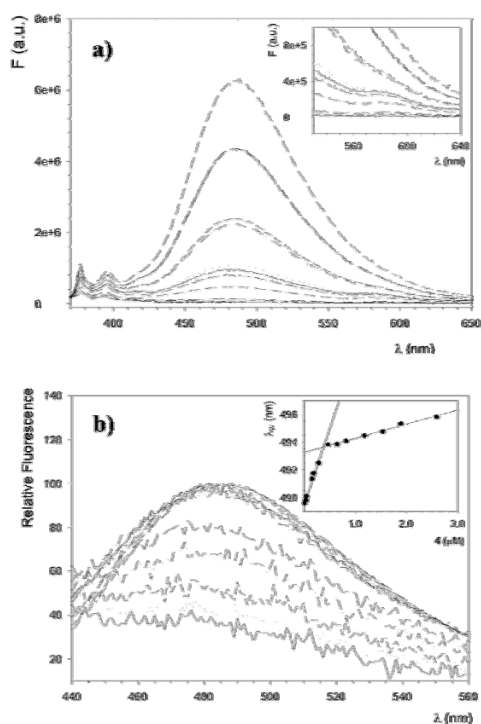
Figure S6. a) Emission fluorescence spectra at increasing concentrations (3×10^{-8}-3×10^{-6} M) of surfactant **4**, all the spectra were recorded at 328 K; inset: portion (550-640 nm) of the emission fluorescence spectra of **4** at increasing concentrations; b) normalized emission fluorescence spectra at increasing concentrations (3×10^{-8}-3×10^{-6} M) of surfactant **4** in the range 440-560 nm; inset: plot of the weighted wavelength (λ_w) of the emission fluorescence spectra versus concentration of surfactant **4**.
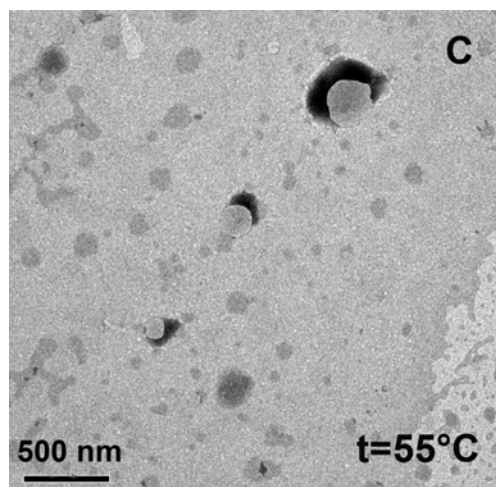
EFTEM measurements

Figure S7. EFTEM images of 10^{-4} M aqueous solution of surfactant **4** at 55°C. The size of the aggregates increasing temperature does not change with respect to 20°C.

References

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