ELECTRONIC SUPPLEMENTARY INFORMATION

for the Article

Aggregation/Disaggregation of Chlorophyll *a* in Model Phospholipid–Detergent Vesicles and Micelles

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Covered Topics

Abbreviations

Chl, **Chl**orophyll;

Chl *a*, **Chl**orophyll ***a***;

DMPC, 1,2-**D**i**m**yristoyl-*sn*-glycero-3**p**hospho**c**holine;

DTAC, **d**odecyl**t**rimethyl**a**mmonium **c**hloride.

1. Turbidity Correction of Electronic Absorption Spectra of Chlorophyll *a* in DMPC–DTAC Media

The absorption bands of Figure 1 of the Main Article are presented after correction of background turbidity, by fitting the original chlorophyll a (Chl *a*) spectra to eq. 1 of the Main Article (see also references [1,2] below). The empirical parameters obtained do not have a straightforward meaning, except for the parameter *c*, which depends on the dimensions of colloidal scattering particles relatively to the wavelength of incident radiation, and their size polydispersity [3]. This parameter is summarized in Table S1 for typical situations.

Table S1. Empirical parameter *c* of eq. 1 (Main Article), used for turbidity correction of the absorption spectra of Chl *a* in the DTAC–DMPC vesicles and micelles

Media	***c***
DMPC liposomes	0.9
Vesicles, D:L = 0.3	2.4
Vesicles, D:L = 1.0	2.4
Vesicles, D:L = 2.0	2.0
Vesicles, D:L = 4.0	3.2
Vesicles, D:L = 6.7	2.3
Micelles, D:L = 20	2.4
Micelles, D:L = 33	3.5

The unexpectedly low value of *c* obtained in DMPC liposomes ($c < 1$) may be explained by the huge size polydispersity of vesicles [3], in agreement with our DLS data (diameters around ≈100–10000 nm) [4]. For D:L ratios ≈0.3–20, *c* is almost constant, at ≈2.0–2.4, meaning that quite large structures (vesicles, cylindrical micelles) exist in these solutions, accordingly to DLS data [4]; the slightly larger *c* value at D:L = 4 (3.2) is an exception: it accounts for *smaller* and *less polydisperse* structures: spontaneous ULVs and disks, which coexist around this D:L ratio and have mean equivalent diameters ≈100 and ≈30 nm, respectively [4]. Only at D:L = 33 *c* approaches the value of 4, typical of very small scattering particles (spherical micelles) [3].

2. Parameters of Electronic Absorption Spectra of Chlorophyll *a* in DMPC–DTAC Media

Table S2 collects the wavelengths at maximum absorption and corresponding molar absorptivities of the chlorophyll *a* Soret and Q bands, in DMPC–DTAC media and in the reference solvent, diethyl ether.

Table S2. Electronic absorption spectral parameters of Chl *a* in pure DMPC liposomes, DMPC–DTAC mixtures at different D:L molar ratios, DTAC micelles and premicelle media, and diethyl ether. The Q and Soret wavelength maxima are denoted λ_Q and λ_S, and the corresponding molar absorptivities (estimated relatively to diethyl ether values) ε_Q and ε_S, respectively

Media	λ_S /nm	λ_Q /nm	$10^{-4}\,\varepsilon_S$ /(M^{-1} cm^{-1})	$10^{-4}\,\varepsilon_Q$ /(M^{-1} cm^{-1})
DMPC liposomes	437	671	4.3	3.4
Mixed vesicles/micelles				
Vesicles, D:L = 0.3	439	673	3.2	2.3
Vesicles, D:L = 1.0	437	671	3.0	2.2
Vesicles, D:L = 2.0	437	672	3.0	2.3
Vesicles, D:L = 4.0	437	671	3.2	2.3
Vesicles, D:L = 6.7	438	671	3.1	2.3
Micelles, D:L = 20	437	670	2.8	2.5
Micelles, D:L = 33	436	670	2.9	2.5
DTAC micelles	436	669	1.8	1.9
Pre-micelle media				
DTAC, 5 mM	—	670	—	0.8
DTAC, 10 mM	437	669	1.3	1.3
DTAC, 20 mM	437	670	1.8	2.1
Diethyl ether	429	661	11.7	8.9

3. Steady-State Fluorescence of Chlorophyll *a* in DTAC Media

Figure S1 illustrates fluorescence emission spectra of Chl *a* in DTAC pre-micelle and micelle media. Comparing with the solvent diethyl ether, the Chl *a* emission intensity is decreased in pre-micelle media at low DTAC concentrations. This enhanced quenching is likely caused by the formation of Chl aggregates, which are less emissive than monomers (see the Main Article, FLIM Imaging subsection). On the other hand, at large DTAC concentrations (in pure or mixed micelles) Chl–Chl interactions within the aggregates are disrupted and Chl monomers, with larger lifetimes, become dominant (Main Article, Fluorescence Lifetimes subsection). The maximum band intensity is a function of the DTAC concentration (inset), showing a marked singularity at 22 ± 1 mM and stabilization afterwards. This value agrees quite well with the *CMC* of DTAC (≈22–22.5 mM) found by conductivity and surface tension measurements [5].

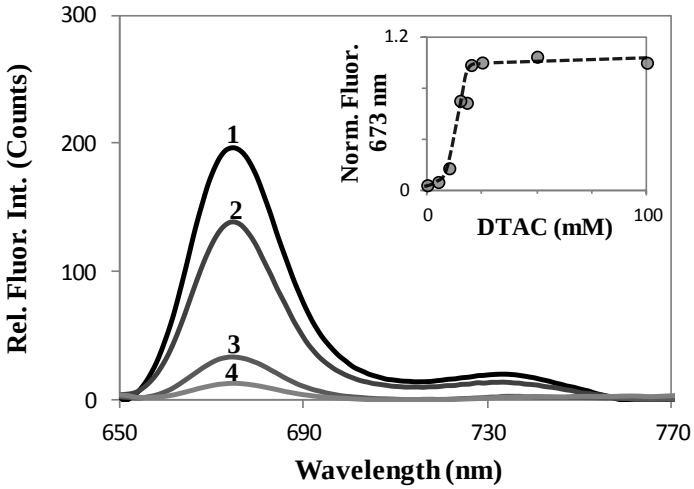

Figure S1. Emission spectra of Chl *a* in: DTAC micelles, at 50 mM DTAC (**1**) and DTAC pre-micelle media, at 15, 10, and 5 mM DTAC (**2**, **3**, and **4**, respectively). **Inset**: Effect of DTAC concentration on the maximum band intensity. Excitation at 638 nm

4. Fluorescence Lifetimes of Chlorophyll *a* in DMPC–DTAC Media

Figure S2 (A,B) illustrates fluorescence emission decays of Chl *a* in diethyl ether, DMPC liposomes, DTAC–DMPC vesicles and micelles, and DTAC micelles. A sum of exponential functions, eq. 2 (Main Article), was reasonably fitted to the decays, with the obtained pre-exponentials (amplitudes) and lifetimes summarized in Table 1 (Main Article). Figure S2 (C,D) further correlates the lifetimes and amplitudes with the D:L molar ratios.

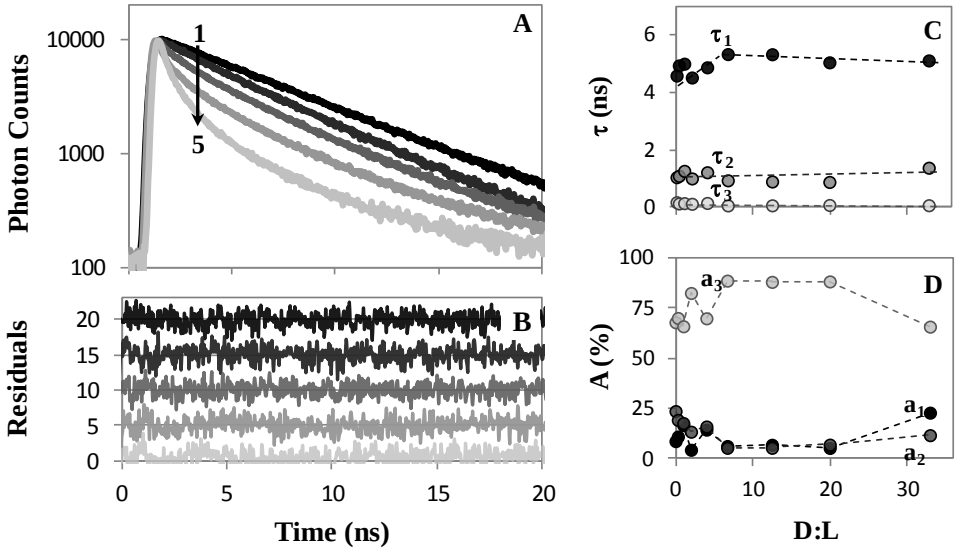

Figure S2. A, emission decays of Chl *a*, and **B**, residuals from their fits, to: a mono-exponential function in diethyl ether (**1**); a bi-exponential function in DTAC micelles (50 mM DTAC, **2**); and a tri-exponential function, in: mixed micelles, D:L = 33 (**3**); mixed liposomes, D:L = 1 (**4**); and DMPC liposomes (**5**). **C**, Lifetimes and **D**, Pre-exponentials obtained for Chl *a* in the DTAC–DMPC mixed system, as a function of D:L: the index **1** refers to monomers; and **2** and **3** to aggregates. Excitation: 638 nm. Emission: 667–722 nm.

Figure S3 (A,B) illustrates fluorescence emission decays of Chl *a* in DTAC pre-micelle media. The calculated pre-exponential and lifetime values are summarized in Table S3, where they are compared with those in pure DTAC micelles. Figure S3 (C,D) further correlates the lifetimes and amplitudes with the D:L molar ratios: the longer lifetime component $\mathbf{a_1}$ (Chl monomers) dominates in micelles, whereas the shorter lifetime component $\mathbf{a_3}$ (Chl aggregates) only appears in pre-micelle media.

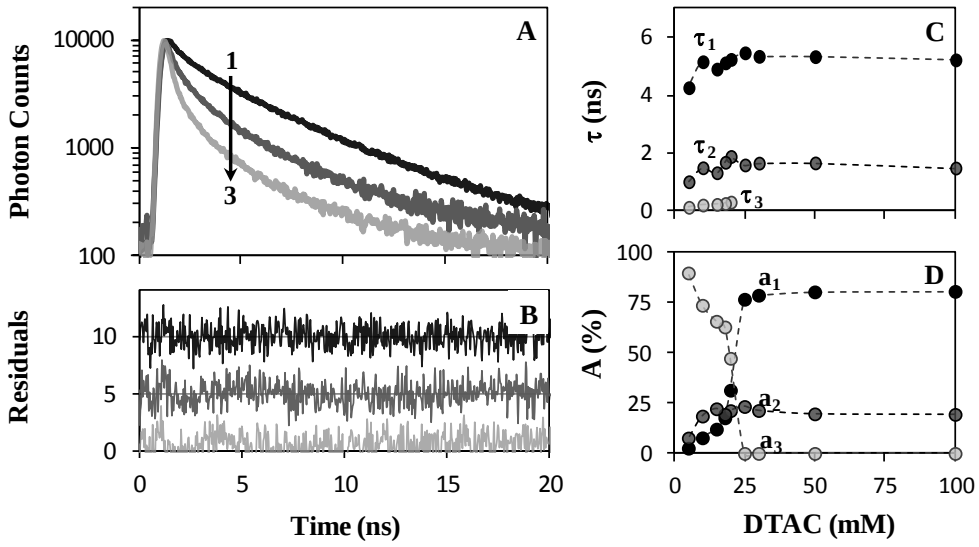

Figure S3. A, Fluorescence decays of Chl-*a*, and **B**, residuals from their fits to a tri-exponential function, in DTAC pre-micelle media, at 20, 10, and 5 mM DTAC (curves **1**, **2**, and **3** respectively). **C** and **D**, Lifetimes and pre-exponentials as a function of the DTAC concentration: the index **1** refers to monomers and **2** and **3** to aggregates. Excitation: 638 nm. Emission: 667–722 nm.

Table S3. Pre-exponentials (***a***) and lifetimes (**τ**) obtained from the analysis of Chl *a* fluorescence decays in DTAC pre-micelle media, as compared with pure DTAC micelles

Solvent Media	a_1 **/%**	τ_1 **/ns**	a_2 **/%**	τ_2 **/ns**	a_3 **/%**	τ_3 **/ns**
Premicelle media						
DTAC, 5 mM	3	4.24	8	0.99	90	0.11
DTAC, 10 mM	8	5.14	19	1.48	74	0.19
DTAC, 20 mM	31	5.22	21	1.87	47	0.29
DTAC micelles	80	5.32	20	1.64	—	—

The multi-exponential decays of Chl *a* in DTAC pre-micelle media evidence the coexistence of monomers and oligomers, with dominance of large oligomers. Three lifetime components were obtained: the *minor* population (≈4–5 ns) corresponds to Chl monomers and the two others (≈1–2; ≈0.1–0.3 ns) to aggregates likely complexed with DTA^+ long ions (FLIM images of these complexes are seen in section 5).

5. FLIM Imaging of Chlorophyll *a* in DTAC Pre-Micelle Media

In pre-micelle media (at 5–15 mM DTAC), large (micro-sized) aggregates where found (Figure S4). Their lifetimes are given in panels A–C (*color scale* at left) and in their corresponding histograms (panels D–F), peaked at ≈4.5 ns. These big structures show irregular and heterogeneous morphologies. They are possibly Chl *a unordered* aggregates complexed with the detergent DTA^+ long ions. The size of these complexes tends to slightly increase with the increase in DTAC amount, up to the formation of micelles; thereafter, complexes completely disaggregate (Main Article, Figure 5E and Lifetime data).

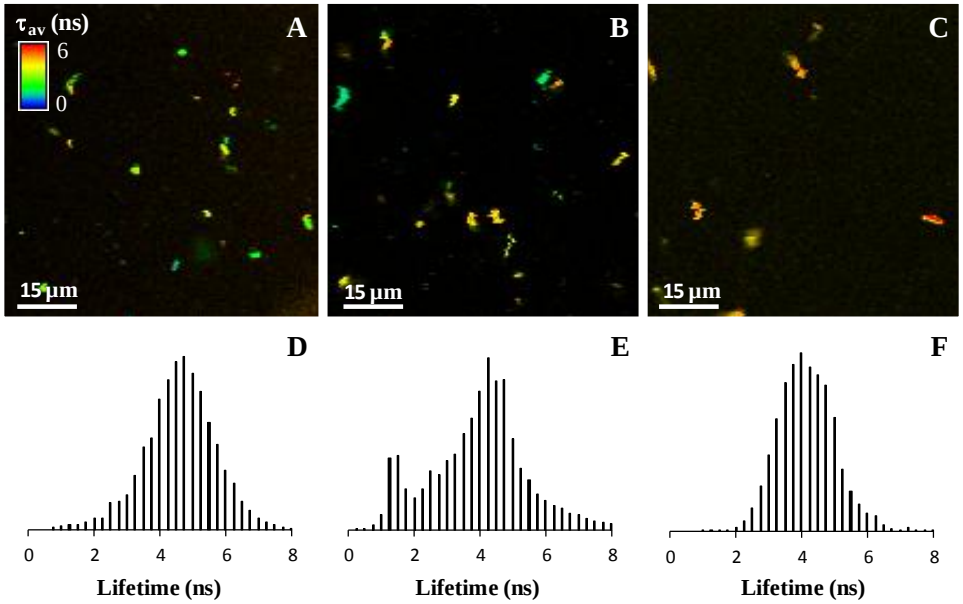

Figure S4. A–C: FLIM images of Chl *a* in DTAC pre-micelle solutions, at 5 (**A**), 10 (**B**), and 15 (**C**) mM DTAC. **D–F**: Normalized lifetime histograms for Chl *a*.

These results corroborate those from fluorescence lifetime measurements (see Table S3).

6. FCS Parameters for Chlorophyll *a* in DMPC–DTAC Media

FCS measurements were performed for Chl *a* incorporated in pure DMPC liposomes, DTAC micelles, and DTAC–DMPC vesicles and micelles. Figure 6 (Main Article) illustrated typical autocorrelation curves (**1**–**5**) and exemplified characteristic size distributions (insets). Herein, Table S4 displays the fitted parameters of the diffusion model; and the calculated equivalent diameters of the colloidal nanostructures, compared with those obtained by previous DLS measurements [4].

Table S4. FCS parameters of the free diffusion model (diffusion time τ_D and diffusion coefficient D) fitted to the experimental autocorrelation curves; and calculated neutral-sphere-equivalent diameters Φ_e [a] of the colloidal structures containing Chl *a*. Equivalent diameters obtained by DLS in the absence of probes Φ_e [b] are given for comparison.

Sample	τ_D [a] /ms	D [a] /(μm^2 s^{-1})	Φ_e [a] /nm	Φ_e [b] /nm
DMPC liposomes	100–6000	0.095–6	100–5000	100–2000
Vesicles, D:L = 1	50–700	0.8–10	50–500	≈110; ≈300
Vesicles, D:L = 2	40–450	1–30	30–350	90–100
Vesicles, D:L = 4	60–230	2–6.5	70–220	≈100
Disks, D:L=4	33 ± 5	17 ± 3	25 ± 4	25–30
Threads, D:L = 12.5	100–750	0.5–5	80–700	30–400; >950
Spherical micelles, D:L = 33	9.4 ± 0.5	60 ± 3	7.1 ± 0.3	≈5.6
DTAC micelles	6.1 ± 0.3	92 ± 4	4.6 ± 0.2	4.0 [c]

[a] This work.

[b] From DLS measurements [4].

[c] From reference [6].

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