Supporting Information

Stable Vesicles Composed of Mono- or Dicarboxylic Fatty Acids and Trimethylammonium Amphiphiles

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1. Supporting Information - Materials and Methods

Ultrafiltration - Calibration of DTAB colorimetric assay

The absorbance of the dye bromothymol blue (0.2mM concentration in assay) was inversely proportional to the DTAB concentration in the samples, i.e., $A_{norm} = A/A_0 \propto 1/c$ where A is the absorbance of the DTAB solution with the quenched dye, A_0 the absorbance of the pure dye solution and c the surfactant concentration. The hypochromic effect commenced after an offset of 0.2mM

DTAB, but above 0.2mM the absorbance could be expressed by the following the equation, which is similar to the equation commonly used for static quenching phenomena (Joseph R. Lakowicz in *Principles of fluorescence spectroscopy*, Vol. 1, Springer 2010, 4th edition, p. 237ff):

$$A_0/A = 1 + a\ (c - 0.2\ mM),$$

where a is the linear coefficient, c the DTAB concentration in mM, A_0 absorbance of the pure dye solution and A the measured absorbance. Figure S1 shows the calibration curve obtained from the average of three experimental series.

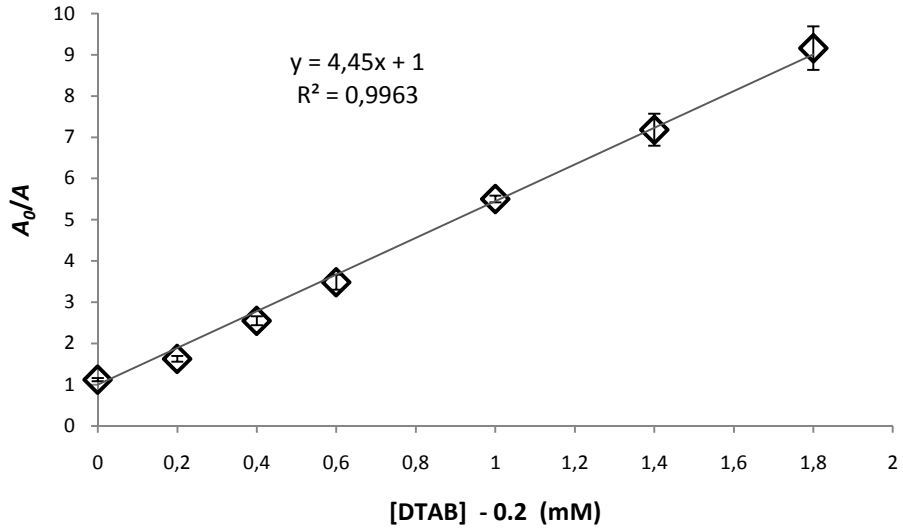

Figure S1: Calibration of the colorimetric assay for DTAB. Linear relation with increasing concentration of DTAB following the equation described above. A is the absorbance of the DTAB solution with the quenched dye, A_0 the absorbance of the pure dye solution. Error bars represent the standard deviation.

DTAB concentrations of all ultrafiltration filtrates were calculated using this calibration curve.

Ultrafiltration - amphiphile quantification control experiments

In order to ensure that during the many steps of the analysis method no bias was introduced, several control experiments were performed.

i) Dynamic Light Scattering. Measurement of the perturbation of the vesicle morphology during ultrafiltration. 20mM mixed amphiphile vesicle samples (400nm pore size, see Materials and Methods in the main manusicript) were measured immediately after extrusion, but before the ultrafiltration procedure and immediately after the ultrafiltration (unfiltrated volume after centrifugation at 13,000 rpm for 3 min) to determine the bulk mean diameter and polydispersity of the vesicles. Each reported value is the average of at least three measurements (see Table S1).

Table S1: Effect of centrifugation on bulk polymorphism. Mean diameter and polydispersity given with standard deviation from four measurements.

Amphiphile system	Centrifugation	Mean diameter (nm)	Polydispersity	Avg. count rate (kcps)
DA / DTAB (20mM total)	Before	113.6 ± 0.8	0.218 ± 0.005	27.5 ± 0.6
	After	122.7 ± 2.3	0.249 ± 0.005	31.4 ± 1.50
$C_{16:0}$-dioic acid/ DTAB (20mM total)	Before	91.7 ± 1.0	0.143 ± 0.010	27.9 ± 0.05
	After	98.2 ± 1.3	0.146 ± 0.010	39.4 ± 0.8

For the DA/DTAB system the mean diameter increased by 8% and the polydispersity by 14%, in the case of $C_{16:0}$-dioic acid/ DTAB, mean diameter and polydispersity increased by 7% and <1%, respectively. The count rate did not show drastic changes, and the observed increase originates from the up-concentration of the total amphiphiles concentration due to the removal of about 25% of the aqueous phase containing amphiphiles at their individual CVC concentrations. Thus, from this data it was concluded that the majority of vesicles were not influenced i.e. remained intact during the ultrafiltration procedure.

ii) Control experiments for the DTAB assay.

The control experiments for the DTAB quantification method give information on the recovery of DTAB after the various steps towards the final assay (Ultrafiltration → Extraction → Assay) and showed the importance of using phosphate buffer instead of Trizma and of performing separating DTAB from the C16:0-dioic acid, despite some loss of material due during the extraction (See Table S2).

Table S2 Control experiments to determine the recovery of the DTAB quantification method. All standards prepared in 0.05M potassium phosphate buffer (apart from the control in 0.1M Trizma buffer). Extractions: 2x 300µL DCM from 200µl acidified sample.

Condition (of 100µl added to assay)	**Content**	**Measured (mM)**	**Recovery%**	**Recovery% relative to "Untreated"**
Untreated	5mM DTAB	4.1	81.6	100.0
Untreated (0.1M Trizma instead)	5mM DTAB	4.2	83.7	102.6
Filtered only (filtrate)	5mM DTAB	3.7	74.5	91.3
Filtered only (concentrate)	5mM DTAB	4.0	79.5	97.6
Extracted + filtered (aqueous phase)	5mM DTAB	3.3	65.6	80.4
Organic phase control	25mM DTAB	6.7	26.9	n/a
Equimolar $C_{16:0}$-dioic acid present (i.e. no extraction)	5mM DTAB/ 5mM $C_{16:0}$-dioic acid	2.7	54.6	67.0

iii) Control experiments for the DA and $C_{16:0}$-dioic acid quantification

The recovery of the fatty acid derivatization and UV-vis detection are reported in Table S3.

Table S3 Recovery of fatty acids by derivatization with p-bromophenacyl bromide.

Entry	**Composition**	**Measured (mM)**	**Recovery (%)**
DA recovery	0.4 mM DA, 50mM phosphate buffer (pH 7.5)	0.358	89.6%
$C_{16:0}$-dioic acid recovery	1.23 mM C:16-dioic acid in ACN	0.85	69%
DTAB interference	5mM DTAB, 50mM phosphate buffer (pH 7.5)	0.014	n/a

2. Supporting Information - Results

Amphiphile bilayer morphologies

The various observed bilayer morphologies dependent on pH and composition are shown in Figure S2 (see caption for individual conditions).

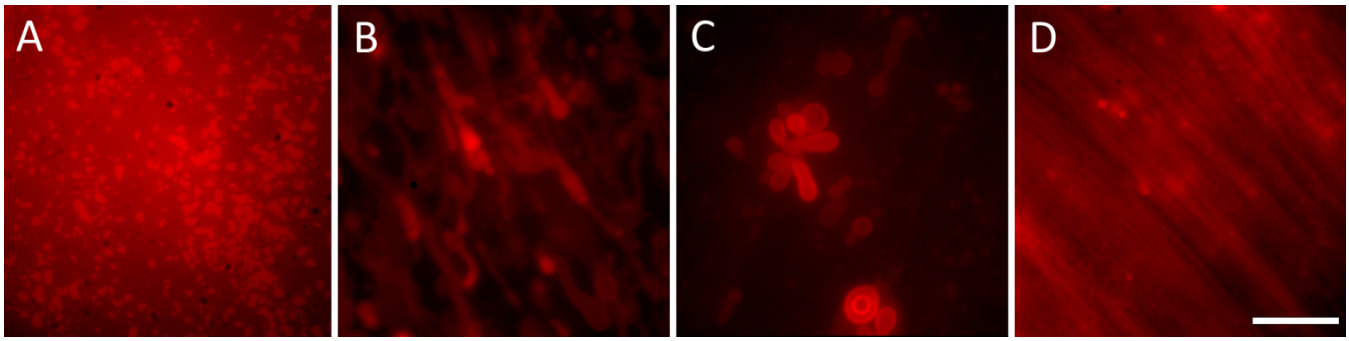

Figure S2. Microscopy images of different bilayer morphologies. Epifluorescence microscopy images obtained from decanoic acid (DA) / decyltrimethylammonium bromide (DTAB) amphiphiles mixtures at various pH and other conditions. **A)** Supported membrane, pH 9.3, 15mM DA/DTAB 1:1. **B)** Mesophase/Tubes, pH 8.16, 20 mM DA/DTAB 1:1, on the same slide also normal vesicles where observed. **C)** Multilamellar and multivesicular vesicles at high concentrations, pH 7.54, 60mM DA/DTAB 1:1. **D)** Fine tubes / hairs, pH 7.25, 60mM DA/DTAB, observed on a slide where the pressure of objective against coverslip created a flow in the liquid.

Encapsulation of HPTS

The epifluorescence microscopy images (Figure S3) show the retention of the negatively charged dye HPTS in vesicles of the $C_{16:0}$-dioic/DTAB system after 10-fold dilution of the external medium.

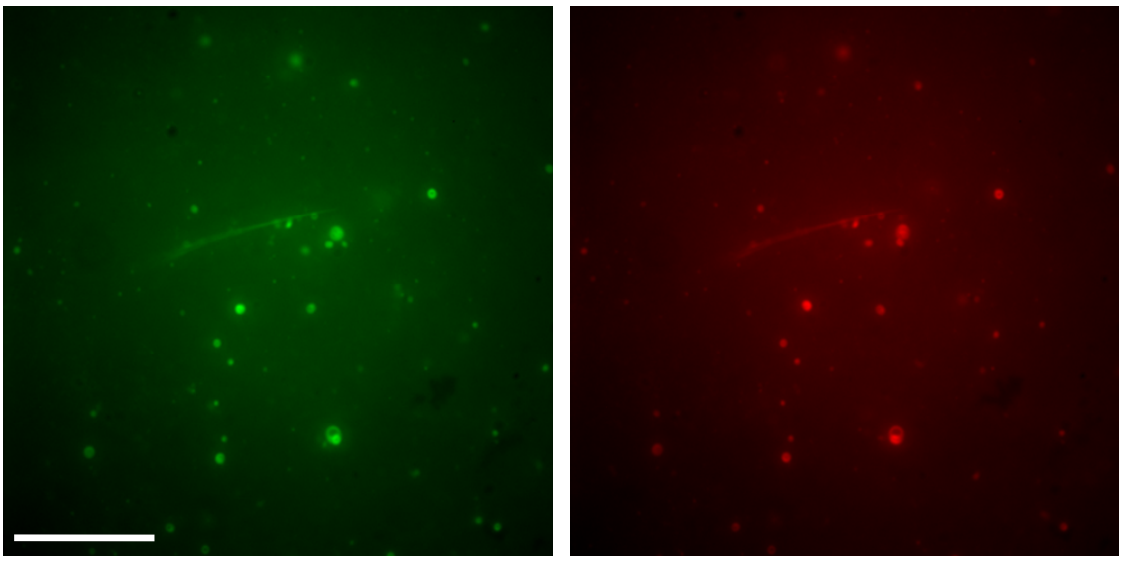

Figure S3. Encapsulation of HPTS in $C_{16:0}$dioic acid/DTAB vesicles. 50 mM of a 1:1 mixture of $C_{16:0}$dioic acid/DTAB vesicles prepared in the presence of 1 mM HPTS and visualized immediately after 1:10 dilution with buffer. Bar = 25 µm. Green, HPTS fluorescence. Red: Nile Red fluorescence.

Langmuir-Blodgett trough

The following graphs show the measured compression isotherm of the four systems studied (Figure S4)

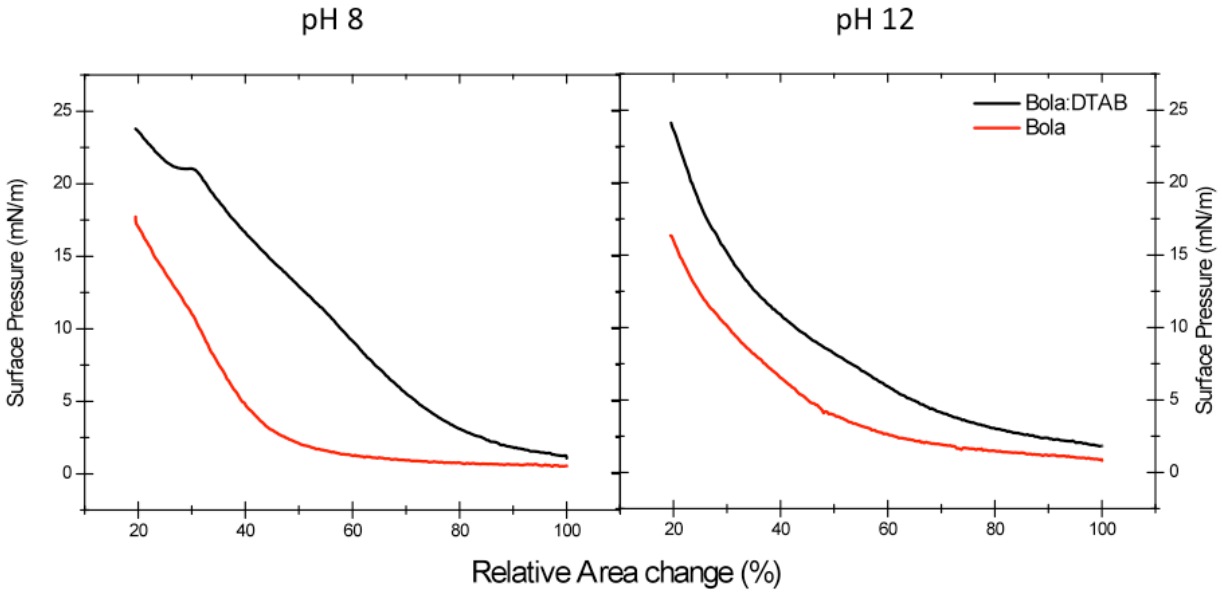

Figure S4 Compression isotherms of the different formulations at different pH

The fitting parameters for the bi-exponential decay of the interfacial film relaxation measurements are listed in Table S4.

Table S4 Bi-exponential decay values obtained after fitting the interfacial film relaxation kinetics graphs for $y = y_0 + A_1e(-x/t_1) + A_2e(-x/t_2)$

		Y_0	A_1	t_1	A_1	t_1
pH 8	Bola:DTAB	77.44±0.06	6.93±0.07	6.29±0.11	15.73±0.04	219.54±1.73
	Bola	71.81±0.11	6.29±0.07	11.35±0.23	21.89±0.08	247.56±2.57
pH 12	Bola:DTAB	52.94±0.08	6.70±0.06	12.44±0.21	39.97±0.05	221.80±1.02
	Bola	65.89±0.08	27.63±0.05	208.14±1.38	6.02±0.06	11.55±0.25

Reduced Chi-Sqr < 0.015

Adj. R-Squared > 0.9995

Membrane composition

The table below lists the raw data for the determination of amphiphile concentration after ultrafiltration, which is plotted in Figure 2.

Table S5: Filtrate compositions of various concentrations: Both experiments performed in 50mM phosphate, pH 7.5.

	$C_{16:0}$-dioic acid/DTAB 1:1 mixture		$C_{16:0}$-dioic acid/DTAB 1:1 mixture	
Concentrations of each amphiphile	DA / mM	DTAB / mM	C16:0-dioic acid/ mM	DTAB/ mM
1 mM	0.40	1.03	0.14	n/a*
2.5 mM	1.26	1.17	0.57	0.98
5mM	1.97	1.98	0.73	1.71
7.5mM	2.13	2.92	0.72	2.41
10mM	2.17	4.29	0.69	3.24

*****below detection limit for DTAB assay.