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Sorption of Cationic Surfactants to Artificial Cell Membranes

Comparing Phospholipid Bilayers with Monolayer Coatings and Molecular Simulations

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Supporting Information for:

Sorption of Cationic Surfactants to Artificial Cell Membranes: Comparing Phospholipid Bilayers with Monolayer Coatings and Molecular Simulations.

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Table S1. Test compounds

Compound	Code	pK _a	logP ^f est.	CMC (mM)	Supplier	CAS number	Purity (%)	Molecular structure
Octylamine	P8	10.7	3.06	400 ^a	Alfa Aesar	111-86-4	99	
Decylamine	P10	10.6	4.12	48 ^a	Sigma-Aldrich	2016-57-1	99.2	
Dodecylamine	P12	10.6	5.18	20 ^b	Sigma-Aldrich	124-22-1	>99.5	
N-methyl-1-octanamine	S8	10.9	3.29		Alfa Aesar	2439-54-5	98	
N-methyl-1-decanamine	S10		4.25		Angene	32509-42-5	95	
N-methyl-1-dodecanamine	S12		5.41		Sigma-Aldrich	7311-30-0	97	
N-hexyl-1-hexylamine	S2-C6		4.88		Sigma-Aldrich	143-16-8	97	
N-octyl-1-octanamine	S2-C8	±11	7.01		Alfa Aesar	1120-48-5	98	
N,N-dimethyl-1-octanamine	T8	±9.9	3.78		Sigma-Aldrich	7378-99-6	95	
N,N-dimethyl-1-decanamine	T10		4.84		TCI	1120-24-7	>93	
N,N-dimethyl-1-dodecanamine	T12	10.0	5.91	0.3 ^c	TCI	112-18-5	>95	
N,N,N-trimethyl-1-octanaminium Br	Q8	N/A		260 ^d	Sigma-Aldrich	2083-68-3	>98	
N,N,N-trimethyl-1-decanaminium Br	Q10	N/A			Sigma-Aldrich	2082-84-0	>98	
N,N,N-trimethyl-1-dodecanaminium Cl	Q12	N/A		60 ^d	Sigma-Aldrich	112-00-5	>99	
N,N,N-trimethyl-1-tetradecanaminium Cl	Q14	N/A		5.6 ^e	Sigma-Aldrich	4574-04-3	>98s	
N-benzyl-N,N-methyl-1-hexanaminium Cl	BAQ6	N/A			Sigma-Aldrich	22559-57-5	>96	
N-benzyl-N,N-methyl-1-octanaminium Cl	BAQ8	N/A			Sigma-Aldrich	959-55-7	>96	
N-benzyl-N,N-methyl-1-decanaminium Cl	BAQ10	N/A			Sigma-Aldrich	63449-41-2	>97	
1-dodecylpyridinium Cl·H ₂ O	PYR12	N/A			Alfa Aesar	207234-02-4	98	

^a ref¹; ^b ref²; ^c ref³; ^d ref⁴; ^e ref⁵; ^f logP values are logK_{OW} values for neutral species estimated by ACD/Labs

Table S2. LC-MS parameters

The interface for the MS-MS was a Turbo Ion spray in positive ionization mode operated at 400 °C, except for P10 which was detected at 300 °C. The following settings were used for all compounds: nebulizer gas (NEB) = 8; collision cell gas (CAD) = 3; collision cell entrance potential (EP) = 10 V; collision cell exit potential (CXP) = 12 V. Compound-specific settings can be found in the table. (CUR = curtain gas; IS = ion spray voltage; DP = declustering potential; FP = focusing potential; CE = collision energy).

Compound	M1 m/z	M3 m/z	CUR	IS (V)	DP (V)	FP (V)	CE (V)
P8	130.1	70.9	7	4500	70	200	16
P10	158.3	57.1	6	4900	70	320	23
P12	186.4	70.9	7	4500	33	186	20
S8	144.1	71	7	4500	60	250	19
S10	172.3	57.1	7	4000	65	380	27
S12	200.2	70.9	7	4500	75	220	24
S2-C6	187.1	103	6	4500	70	400	22
S2-C8	243.4	131	6	4500	70	400	27
T8	158.4	46	7	4500	80	200	30
T10	185.9	57.1	7	4500	70	350	33
T12	214.2	57.1	7	4500	64	195	35
Q8	172.3	60	7	4500	43	292	34
Q10	200.3	60	8	3200	51	300	27
Q12	228.3	60	7	4500	58	292	39
Q14	256.5	60	7	4500	58	292	44
BAQ6	220	91	9	5500	38	292	33
BAQ8	248.2	91	9	5500	45	292	45
BAQ10	276.4	90.9	7	4500	51	292	47
PYR12	248.4	80.1	7	4500	45	270	33

Table S3. Overview of K_{MW} values and associated data for tested chemicals

	Conc. Range (buffer, μM)	Glass binding in ref.	$\log K_{MW}$ (\pm s.e.) TRANSIL pH7.4	N	95% c.i.	$\log K_{MW(IAM)}$ IAM-HPLC pH5.0 ^a corrected (nr. k_{IAM} included)	$\log K_{DMPC-W}$ COSMOmic (cation) ^b incl. offset	maximum $\log K_{DMPC-W}$ difference between conformers (nr. conf.)	$\log K_{MW}$ ^c via $\log K_{OW}$ (neutral)
P8	0.11 - 13.2	25%	3.10 ± 0.03	12	3.04-3.17	3.10*	3.31	0.78 (5)	3.21
P10	0.010 - 2.3	29%	4.30 ± 0.04	12	4.23-4.38	4.35 (5)	4.43	0.84 (5)	4.28
P12	0.0013 - 0.036	57%	5.58 ± 0.04	11	5.49-5.66	-	5.61	0.35 (2)	5.35
S8	1.4 - 12.9	n.d.	2.76 ± 0.01	6	2.73-2.79	2.80*	2.61	0.87 (7)	3.44
S10	0.110 - 6.8	26%	3.98 ± 0.03	11	3.92-4.03	4.07 (7)	3.74	0.35 (2)	4.41
S12	0.0055 - 0.15	46%	5.39 ± 0.01	23	5.37-5.42	5.28*	4.88	0.46 (3)	5.58
S2-C6	0.022 - 0.71	5%	3.15 ± 0.02	14	3.10-3.20	3.35 (6)	2.37	0.35 (6)	5.05
S2-C8	0.074 - 1.26	-	4.65 ± 0.02	6	4.61-4.69	5.51 (6)	3.94	0.67 (4)	7.20
T8	2.46 - 17	n.d.	2.35 ± 0.02	6	2.31-2.40	2.35*	2.13	0.76 (5)	3.94
T10	0.035 - 6.3	26%	3.65 ± 0.02	15	3.61-3.68	3.59 (7)	3.30	0.78 (4)	5.01
T12	0.0026 - 0.20	46%	5.30 ± 0.02	12	5.25-5.34	-	4.36	0.44 (3)	6.09
Q8	1.78 - 27.5	n.d.	2.18 ± 0.04	12	2.10-2.26	2.07*	1.78	0.37 (2)	-
Q10	0.054 - 10.7	n.d.	3.34 ± 0.03	12	3.28-3.40	3.33 (7)	2.90	0.42 (2)	-
Q12	0.031 - 1.2	20%	4.35 ± 0.02	9	4.32-4.39	4.46 (6)	4.07	(1)	-
Q14	0.0052 - 0.180	56%	5.46 ± 0.03	9	5.39-5.54	-	5.21	(1)	-
BAQ6	0.20 - 4.3	n.d.	2.12 ± 0.02	8	2.08-2.16	2.31*	1.47	0.34 (4)	-
BAQ8	0.34 - 3.8	n.d.	3.11 ± 0.01	7	3.08-3.15	3.19*	2.56	0.44 (3)	-
BAQ10	0.010 - 2.0	39%	4.01 ± 0.02	12	3.97-4.06	4.36*	3.65	0.43 (2)	-
PYR12	0.0074 - 0.310	51%	4.89 ± 0.03	10	4.84-4.95	-	4.37	(1)	-

^a $K_{MW(IAM)}$ is based on the retention capacity factor for the Immobilized Artificial Membrane column (k_{IAM}) \times 18.9 phase ratio (water:phospholipids), and corrected for the $\delta_{IAM-SSLM}$ corrective increments determined in this study: +0.78 log units for 1^o amines, +0.48 log units for 2^o amines, -0.03 log units for 3^o amines, and -0.11 log units for QACs. Data with * are from earlier work (ref⁶).

^b COSMOmic calculations were performed with the same DMPC input structures and membrane potential settings as recommended by Bittermann et al. (ref⁷), with a recommended cation offset factor of -0.4 log units. Values are weighted averages of the most relevant conformers.

^c using the regression from Endo et al. (ref⁸): $\log K_{MW} = 1.01 \times \log K_{OW} + 0.12$. $\log K_{OW}$ values are predicted by ACD/Labs.

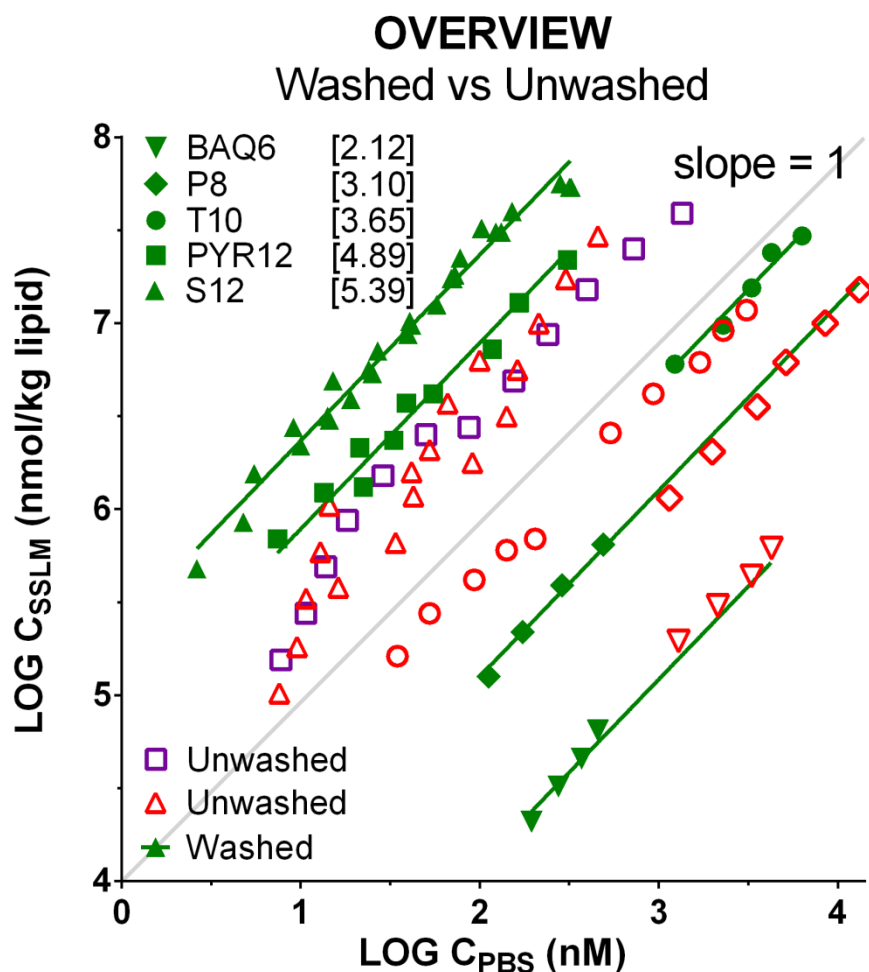


Figure S1. Influence of D_{MW} on significance of leaked phospholipids in medium

A series of cationic surfactants with different D_{MW} values were spiked to:

- A. HPLC autosampler vials that contained SSLM beads in their original test medium (transferred from the 96 well plate – “unwashed”, though diluted by a factor of 2);
- B. HPLC autosampler vials that contained SSLM beads without the original test medium (transferred from the 96 well plate – centrifuged, removal of supernatant and addition of fresh test medium – “washed”).

In accordance with the data modeled elsewhere (supporting information Figure S4), there is no influence of this washing step up to a $\log D_{MW}$ of 3.65 (e.g. the surfactant T10 plotted here).

For S12 ($\log D_{MW}$ of 5.39) the influence of renewing the test medium with fresh PBS, and thus removing the presence of leaked phospholipids, is up to factor 10 higher D_{MW} values and consistent linear isotherms instead of variable/nonlinear isotherms for unwashed beads.

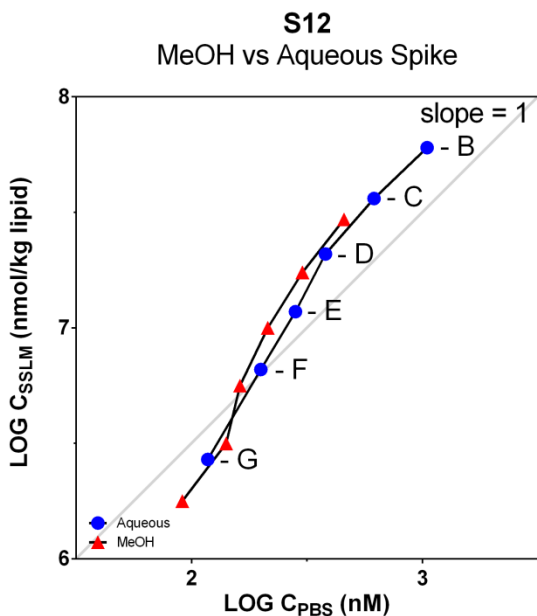


Figure S2. Comparison between aqueous spike solution and spike solution in methanol for N-methyldodecylamine (S12).

This figure indicates that any influence of the composition of the spiking solution is minimal, if present at all, and no explanation for the non-linear shape of the sorption isotherm. Two SSLM test series were compared, of which one was spiked directly with a methanol dosing standard and one was spiked using a dosing standard made up of an aqueous solution. Both dosing standards did not have the same starting concentration, hence the downward shift of the isotherm based on the methanol-spiked series. The sorbent dilution series follow Vials B (lowest SSLM) – G (highest SSLM).

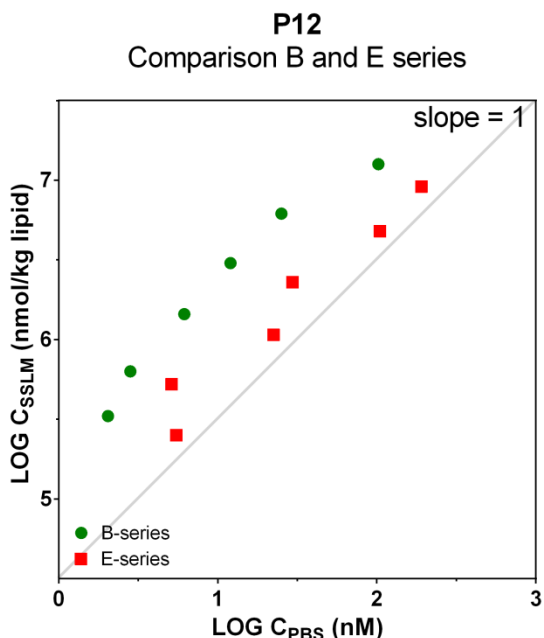


Figure S3. Comparison between B- and E-series for dodecylamine (P12), for which two constant SSLM amounts were spiked with six serially diluted stock solutions in methanol.

This figure shows that when a consistent amount of TRANSIL beads were used in a series spiked with different concentrations of surfactant, a linear isotherm readily fits the data for each series. The B-series has the lowest bead content in a standard row of the 2096 type well plate; the E-series has a 5.8 times higher bead content. Conform the nonlinear relationship within the standard sorbent dilution series (Figure S1), the D_{MW} for the E-series is consistently lower than for the B-series.

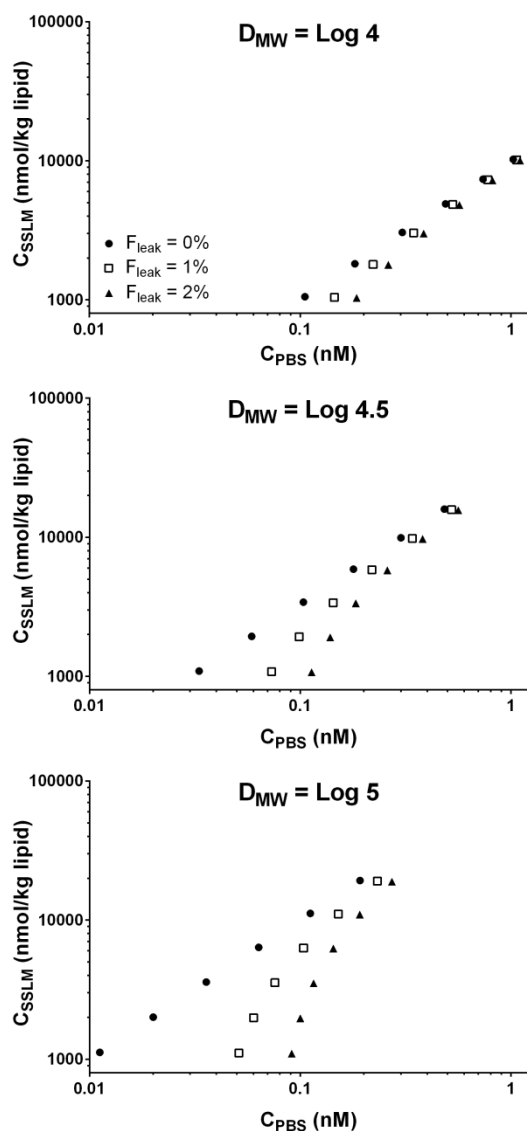


Figure S4. Overview of the predicted influence of phospholipid leakage (0%, 1% and 2% leakage from the SSLM material), at three different sorption affinities in the range $\log D_{MW}$ 4-5.

The modeled values clearly show negligible deviations for a chemical with a $\log D_{MW}$ of 4 at the vials with the lowest SSLM material (highest C_{PBS}), up to a factor of 2 at the highest SSLM material (lowest C_{PBS}) in the case of 2% leakage.

For a chemical with a $\log D_{MW}$ of 4.5 there is still negligible effect of leakage up to 2% lipids at the vials with the lowest SSLM material (highest C_{PBS}), but this increases to a factor of 3 and factor of 8 at the highest SSLM material (lowest C_{PBS}) in the case of 1% and 2% leakage, respectively.

For a chemical with a $\log D_{MW}$ of 5 there is still negligible effect of leakage up to 2% lipids at the vials with the lowest SSLM material (highest C_{PBS}), but this increases to more than a factor of 3 at the highest SSLM material (lowest C_{PBS}) in the case of only 1% leakage, and a factor of 10 with 2% lipids leaking into the test medium.

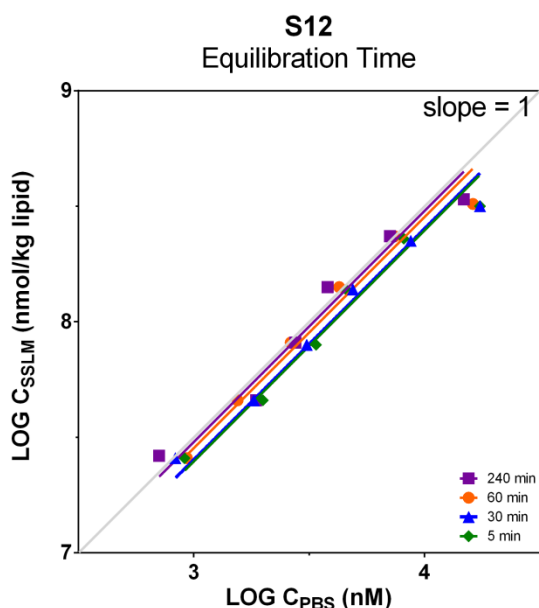


Figure S5. Influence of incubation time on measured D_{MW} for N-methyldodecylamine (S12).

The results presented in this figure prove that incubation time has a very minor, if any, effect on measured D_{MW} . Four series of sorbent dilution series were placed on a roller mixer, each series spiked with the same methanol stock solution. The four different vials in each series were centrifuged (10 minutes) after 5, 30, 60 or 240 minutes on the roller mixer. No statistically significant difference in D_{MW} was observed when fitting with a log-linear sorption isotherm with a fixed slope of 1.

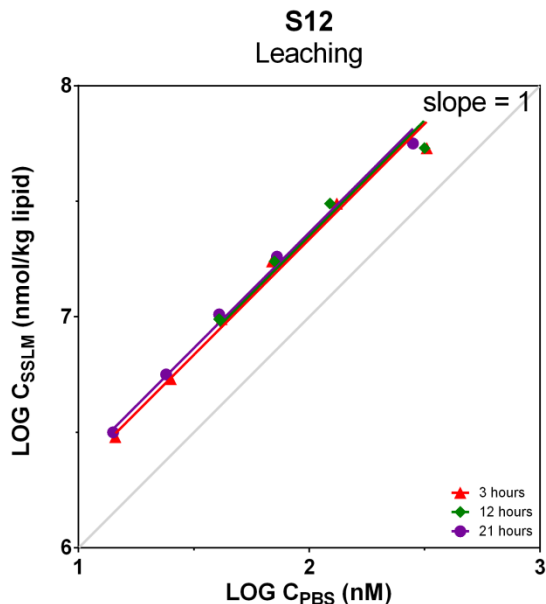


Figure S6. Influence of storing centrifuged samples at room temperature in the autosampler on leaching of lipids, determined by D_{MW} for N-methyldodecylamine (S12).

One series of beads was spiked (after washing as described in M&M) and left for 3 hours at room temperature before centrifugation and measurement. Vials with beads were then left at room temperature and measured again after a total of 12 and 21 hours at room temperature. The results between the three measurements are virtually identical, especially considering analytical uncertainty of LC-MS/MS measurements in general, indicating that lipids do not leak into the medium of the autosampler vial for centrifuged samples, as this would have increased the apparent C_{PBS} .

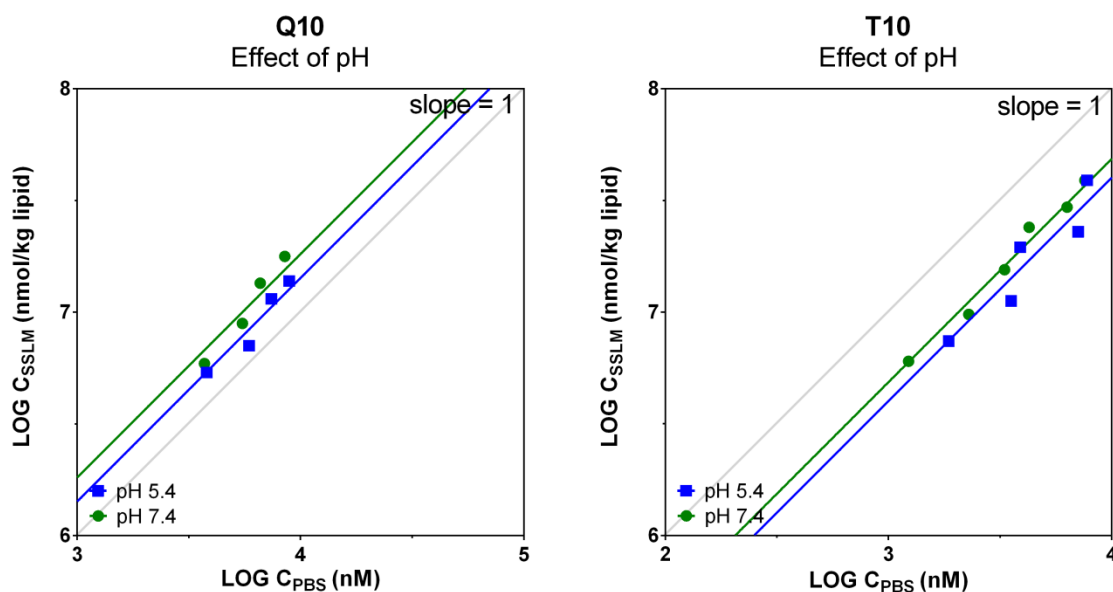


Figure S7. Influence of neutral fraction on D_{MW} estimates

Comparison of a quaternary and tertiary amine at pH 5.4 and pH 7.4 provides evidence that there is negligible contribution of the neutral fraction of T10 at pH 7.4, where the tertiary amine T10 is >99.5% ionic ($pK_a \sim 10$, see Table S1). Q10 is of course 100% ionic at both pH values.

At pH 5.4 the neutral fraction should be 100-fold lower than at pH 7.4. Since only a slight (not statistically significant) decrease of 0.1 log units in D_{MW} is observed for both amines, virtually identical for a quaternary amine that – by definition – is not susceptible to contribution of a pH-dependent neutral fraction, any contribution of the small neutral fraction present at pH 7.4 can be assumed to be trivial.

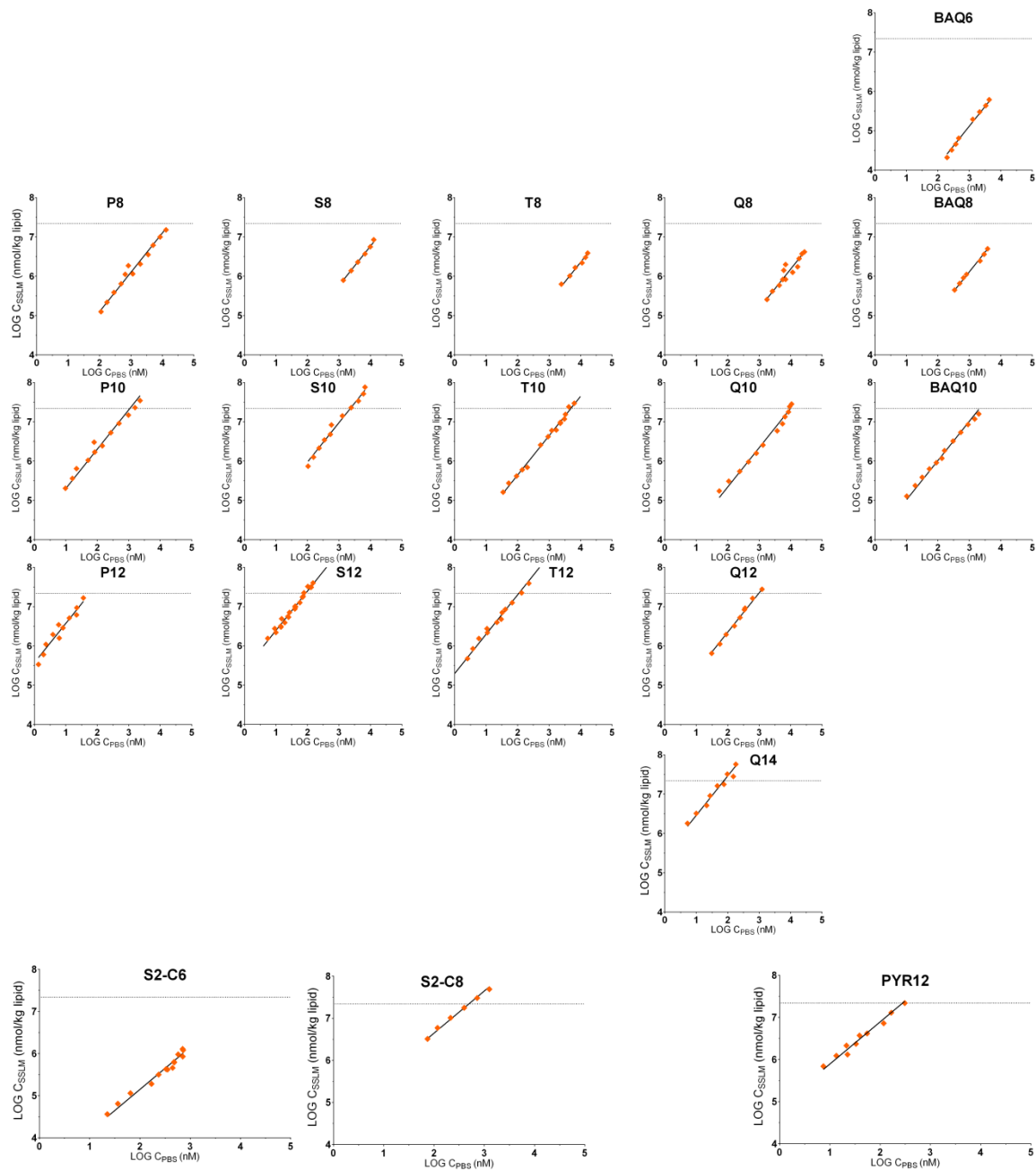


Figure S8. Matrix of all SSLM sorption isotherm plots.

This matrix contains all data points and all isotherms used to calculate the D_{MW} values reported in the tables and manuscript. Both axes are identical for every graph. The dotted line at a sorbed concentration of approximately log 7.3 (nmol/kg lipid) indicates the maximum liposome loading as established in the main text.

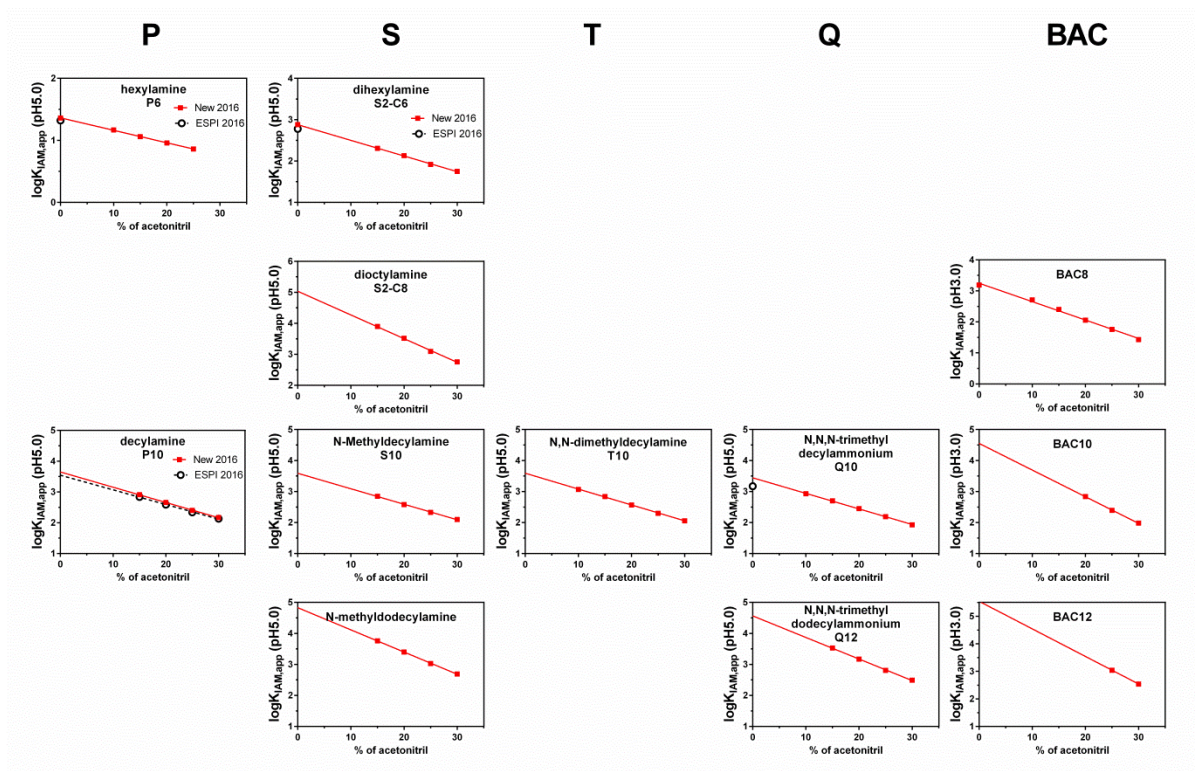


Figure S9. Matrix of all solvent series measurements with IAM-HPLC

Performed in this study, and overlapping IAM-HPLC data from a previous study (ref ⁶). The BAC series were performed previously (ref ⁶).

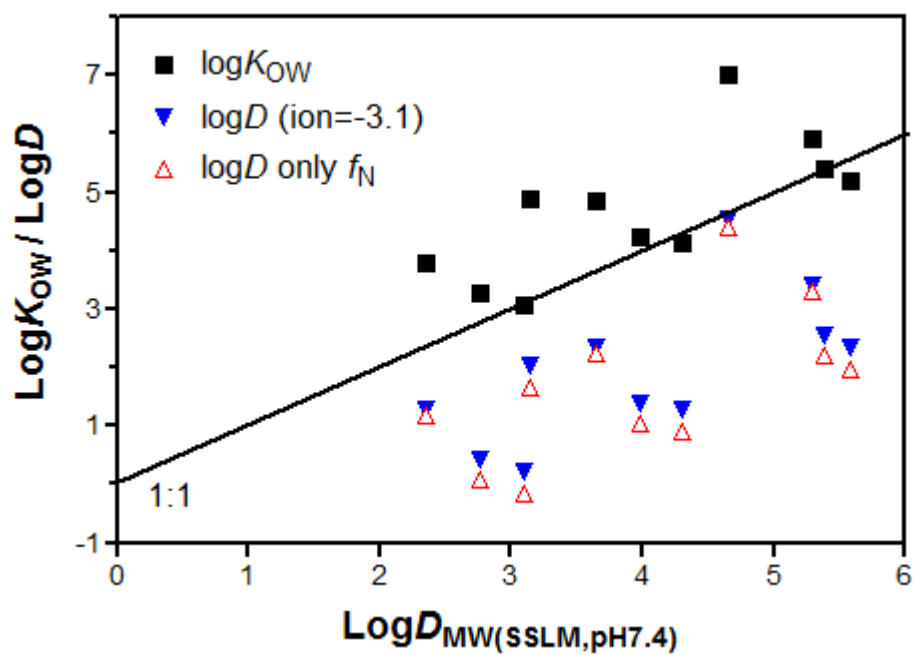


Figure S10. Comparison between $\text{log}P/\text{log}D$ and the sorption affinity to bilayer membrane for ionizable cationic surfactants. $\text{Log}P$ values are taken from ACD/Labs.

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