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Fatty acids' double role in the prebiotic formation of a hydrophobic dipeptide

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Electronic supporting information (ESI)

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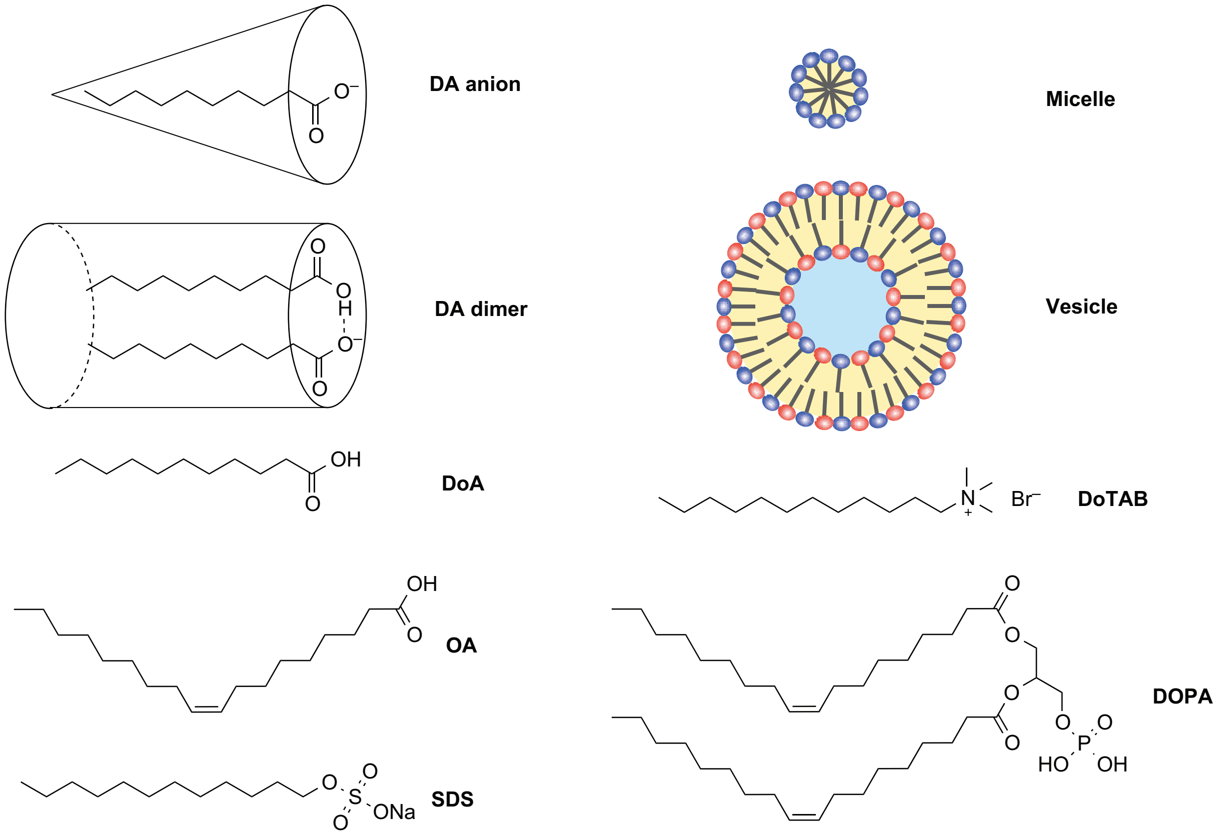


Fig. S1 Decanoic acid structure and self-assembly properties, together with some of the additional amphiphiles and surfactants employed in this work. The overall size of polar heads (including solvation shell) compared to that of hydrophobic tails induces different supramolecular aggregation behaviours.

Table S1 Total yield, measured by the relative HPLC areas of peptides products (detection at 273 nm), and diastereoselectivity (*d.r.* homochiral vs. heterochiral) in the reactions carried out in aqueous buffers from Ac-Tyr(Me) oxazolone (1mM) in the presence of nucleophiles (5 mM) and various amphiphiles/surfactants at room temperature. Given the large variety of conditions tried, most measurements were only performed once, so the errors were calculated as coefficients of variation: 13 % for pH 6.5, 19 % for pH 7.2, 12 % for pH 8 and 18 % for pH 9. This estimation was made from all the control samples, without amphiphile, at each pH, making use of the values of *d.r.* (since the yield is much more sensitive to small variations in the amount of nucleophile and to the degree of purity of the oxazolone in the stock solution).

Surfactant type	Concentration (mM)	Buffer (pH)	Nucleophile	Total yield (%)	*d.r.*
Decanoic Acid	0	100 mM MES (6.5)	H-Leu-NH_2	8.9	1.30
Decanoic Acid	2	100 mM MES (6.5)	H-Leu-NH_2	10.5	1.43
Decanoic Acid	4	100 mM MES (6.5)	H-Leu-NH_2	10.1	1.38
Decanoic Acid	6	100 mM MES (6.5)	H-Leu-NH_2	10.5	1.31
Decanoic Acid	8	100 mM MES (6.5)	H-Leu-NH_2	10.9	1.52
Decanoic Acid	10	100 mM MES (6.5)	H-Leu-NH_2	10.7	1.13
Decanoic Acid	15	100 mM MES (6.5)	H-Leu-NH_2	20.7	0.66
Decanoic Acid	25	100 mM MES (6.5)	H-Leu-NH_2	28.6	0.62
Decanoic Acid	50	100 mM MES (6.5)	H-Leu-NH_2	29.9	0.57
Decanoic Acid (x3)	0	100 mM MOPS (7.2)	H-Leu-NH_2	21.3	1.81
Decanoic Acid (x3)	10	100 mM MOPS (7.2)	H-Leu-NH_2	20.5	1.63
Decanoic Acid (x3)	15	100 mM MOPS (7.2)	H-Leu-NH_2	20.0	1.44
Decanoic Acid (x3)	18	100 mM MOPS (7.2)	H-Leu-NH_2	22.5	1.16
Decanoic Acid (x3)	20	100 mM MOPS (7.2)	H-Leu-NH_2	29.0	0.74
Decanoic Acid (x3)	24	100 mM MOPS (7.2)	H-Leu-NH_2	36.8	0.53
Decanoic Acid (x3)	28	100 mM MOPS (7.2)	H-Leu-NH_2	42.8	0.44
Decanoic Acid (x3)	30	100 mM MOPS (7.2)	H-Leu-NH_2	43.4	0.42
Decanoic Acid (x3)	50	100 mM MOPS (7.2)	H-Leu-NH_2	42.8	0.37
Decanoic Acid	0	100 mM PIPPS (9)	H-Leu-NH_2	3.2	2.60
Decanoic Acid	10	100 mM PIPPS (9)	H-Leu-NH_2	3.0	2.19
Decanoic Acid	15	100 mM PIPPS (9)	H-Leu-NH_2	3.6	2.60
Decanoic Acid	18	100 mM PIPPS (9)	H-Leu-NH_2	3.5	2.03
Decanoic Acid	20	100 mM PIPPS (9)	H-Leu-NH_2	3.3	2.17
Decanoic Acid	24	100 mM PIPPS (9)	H-Leu-NH_2	2.5	1.27
Decanoic Acid	28	100 mM PIPPS (9)	H-Leu-NH_2	3.3	2.25
Decanoic Acid	30	100 mM PIPPS (9)	H-Leu-NH_2	3.2	1.98
Decanoic Acid	50	100 mM PIPPS (9)	H-Leu-NH_2	3.26	1.86
Decanoic Acid / Decanol	0 / 0	100 mM MOPS (7.2)	H-Leu-NH_2	31.5	2.02
Decanoic Acid / Decanol	10 /20	100 mM MOPS (7.2)	H-Leu-NH_2	33.5	1.16
Decanoic Acid / Decanol	15 /15	100 mM MOPS (7.2)	H-Leu-NH_2	31.0	1.12
Decanoic Acid / Decanol	20 /10	100 mM MOPS (7.2)	H-Leu-NH_2	34.0	1.02
Dodecanoic Acid	0	100 mM MES (6.5)	H-Leu-NH_2	17.1	1.69
Dodecanoic Acid	1	100 mM MES (6.5)	H-Leu-NH_2	19.9	1.89

Dodecanoic Acid	5	100 mM MES (6.5)	H-Leu-NH$_2$	24.3	1.28
Dodecanoic Acid	10	100 mM MES (6.5)	H-Leu-NH$_2$	33.5	0.74
Dodecanoic Acid	20	100 mM MES (6.5)	H-Leu-NH$_2$	23.9	1.92
Dodecanoic Acid	0	100 mM MOPS (7.2)	H-Leu-NH$_2$	18.7	1.58
Dodecanoic Acid	1	100 mM MOPS (7.2)	H-Leu-NH$_2$	17.1	1.69
Dodecanoic Acid	5	100 mM MOPS (7.2)	H-Leu-NH$_2$	23.8	0.84
Dodecanoic Acid	10	100 mM MOPS (7.2)	H-Leu-NH$_2$	27.6	0.61
Dodecanoic Acid	20	100 mM MOPS (7.2)	H-Leu-NH$_2$	32.8	0.51
Dodecanoic Acid	0	100 mM MOPS (8)	H-Leu-NH$_2$	11.0	1.30
Dodecanoic Acid	1	100 mM MOPS (8)	H-Leu-NH$_2$	13.9	1.40
Dodecanoic Acid	5	100 mM MOPS (8)	H-Leu-NH$_2$	33.4	0.75
Dodecanoic Acid	10	100 mM MOPS (8)	H-Leu-NH$_2$	35.7	0.62
Dodecanoic Acid	20	100 mM MOPS (8)	H-Leu-NH$_2$	34.8	0.54
Dodecanoic Acid	0	100 mM MOPS (9)	H-Leu-NH$_2$	12.2	2.00
Dodecanoic Acid	1	100 mM MOPS (9)	H-Leu-NH$_2$	12.1	2.01
Dodecanoic Acid	5	100 mM MOPS (9)	H-Leu-NH$_2$	11.5	1.94
Dodecanoic Acid	10	100 mM MOPS (9)	H-Leu-NH$_2$	13.2	1.52
Dodecanoic Acid	20	100 mM MOPS (9)	H-Leu-NH$_2$	16.7	0.89
Dodecanoic Acid	40	100 mM MOPS (9)	H-Leu-NH$_2$	21.5	0.50
Oleic Acid	0	100 mM MOPS (8)	H-Leu-NH$_2$	25.1	1.90
Oleic Acid	0.5	100 mM MOPS (8)	H-Leu-NH$_2$	25.3	1.81
Oleic Acid	1	100 mM MOPS (8)	H-Leu-NH$_2$	26.1	1.67
Oleic Acid	1.5	100 mM MOPS (8)	H-Leu-NH$_2$	24.3	1.80
Oleic Acid	2	100 mM MOPS (8)	H-Leu-NH$_2$	24.1	1.80
Oleic Acid	5	100 mM MOPS (8)	H-Leu-NH$_2$	24.8	1.69
Oleic Acid	10	100 mM MOPS (8)	H-Leu-NH$_2$	26.3	1.44
Oleic Acid	20	100 mM MOPS (8)	H-Leu-NH$_2$	30.4	1.02
Oleic Acid	40	100 mM MOPS (8)	H-Leu-NH$_2$	32.1	0.84
Oleic Acid	0	100 mM PIPPS (9)	H-Leu-NH$_2$	15.1	2.11
Oleic Acid	0.5	100 mM PIPPS (9)	H-Leu-NH$_2$	16.0	2.00
Oleic Acid	1	100 mM PIPPS (9)	H-Leu-NH$_2$	16.2	1.91
Oleic Acid	1.5	100 mM PIPPS (9)	H-Leu-NH$_2$	16.7	1.77
Oleic Acid	2	100 mM PIPPS (9)	H-Leu-NH$_2$	17.2	1.70
Oleic Acid	5	100 mM PIPPS (9)	H-Leu-NH$_2$	19.1	1.34
Oleic Acid	10	100 mM PIPPS (9)	H-Leu-NH$_2$	22.6	1.04
Oleic Acid	20	100 mM PIPPS (9)	H-Leu-NH$_2$	26.5	0.77
Oleic Acid	40	100 mM PIPPS (9)	H-Leu-NH$_2$	33.2	0.57
DOPA	0	100 mM MOPS (8)	H-Leu-NH$_2$	17.5	2.02
DOPA	0.5	100 mM MOPS (8)	H-Leu-NH$_2$	17.7	2.06
DOPA	1	100 mM MOPS (8)	H-Leu-NH$_2$	17.9	1.61
DOPA	1.5	100 mM MOPS (8)	H-Leu-NH$_2$	17.0	1.55
DOPA	2	100 mM MOPS (8)	H-Leu-NH$_2$	17.3	1.51
DOPA	2.5	100 mM MOPS (8)	H-Leu-NH$_2$	18.5	1.28
DOPA	3	100 mM MOPS (8)	H-Leu-NH$_2$	19.4	1.41
DOPA	10	100 mM MOPS (8)	H-Leu-NH$_2$	20.1	1.07

DoTAB	0	100 mM MOPS (8)	H-Leu-NH$_2$	19.5	2.00
DoTAB	10	100 mM MOPS (8)	H-Leu-NH$_2$	16.7	1.69
DoTAB	20	100 mM MOPS (8)	H-Leu-NH$_2$	14.4	1.68
DoTAB	40	100 mM MOPS (8)	H-Leu-NH$_2$	13.1	1.65
SDS	0	100 mM PIPPS (9)	H-Leu-NH$_2$	11.8	2.01
SDS	2.5	100 mM PIPPS (9)	H-Leu-NH$_2$	12.0	1.88
SDS	5	100 mM PIPPS (9)	H-Leu-NH$_2$	12.3	1.75
SDS	8	100 mM PIPPS (9)	H-Leu-NH$_2$	12.5	1.56
SDS	10	100 mM PIPPS (9)	H-Leu-NH$_2$	12.6	1.45
SDS	15	100 mM PIPPS (9)	H-Leu-NH$_2$	13.10	1.27
SDS	20	100 mM PIPPS (9)	H-Leu-NH$_2$	13.5	1.15
SDS	30	100 mM PIPPS (9)	H-Leu-NH$_2$	14.0	0.99
SDS	50	100 mM PIPPS (9)	H-Leu-NH$_2$	14.0	0.85
Oleic Acid	0	100 mM MOPS (8)	Glycine	54.7	-
Oleic Acid	1.5	100 mM MOPS (8)	Glycine	62.4	-
Oleic Acid	5	100 mM MOPS (8)	Glycine	57.6	-
Oleic Acid	0	100 mM MOPS (8)	Alanine	31.4	3.76
Oleic Acid	1.5	100 mM MOPS (8)	Alanine	32.9	2.89
Oleic Acid	5	100 mM MOPS (8)	Alanine	35.0	2.27
Oleic Acid	0	100 mM MOPS (8)	Leucine	25.8	1.52
Oleic Acid	1.5	100 mM MOPS (8)	Leucine	23.7	1.41
Oleic Acid	5	100 mM MOPS (8)	Leucine	21.5	1.31
DoTAB	0	100 mM MOPS (8)	Leucine	11.5	1.27
DoTAB	10	100 mM MOPS (8)	Leucine	11.5	1.27
DoTAB	20	100 mM MOPS (8)	Leucine	12.3	1.16
DoTAB	40	100 mM MOPS (8)	Leucine	5.81	0.93

Table S2 Total yield, measured by the relative HPLC areas of Ac-Tyr(Me)-Leu-NH_2 peptides products (detection at 273 nm), and diastereoselectivity (*d.r.* homochiral vs. heterochiral) of the reactions carried out in organic solvents from Ac-Tyr(Me) oxazolone (1mM) in the presence of 5 mM H-Leu-NH_2 and different carboxylic acid reagents at room temperature.

Carboxylic acid	Conc. / mM	Organic Solvent	Time of reaction	Total yield %	*d.r.*
Acetic Acid (x2)	0	CH_2Cl_2	1 hour	17.4	0.84
Acetic Acid (x2)	2	CH_2Cl_2	1 hour	60.5	0.39
Acetic Acid (x2)	5	CH_2Cl_2	1 hour	79.2	0.42
Acetic Acid (x2)	10	CH_2Cl_2	1 hour	86.2	0.49
Decanoic Acid	0	CH_2Cl_2	1 hour	16.6	0.79
Decanoic Acid	2	CH_2Cl_2	1 hour	65.5	0.41
Decanoic Acid	4	CH_2Cl_2	1 hour	81.4	0.47
Decanoic Acid	10	CH_2Cl_2	1 hour	86.9	0.54
Acetic Acid	0	CH_3CN	1 hour	2.7	0.62
Acetic Acid	2	CH_3CN	1 hour	15.7	0.38
Acetic Acid	5	CH_3CN	1 hour	29.5	0.40
Acetic Acid	10	CH_3CN	1 hour	45.3	0.44
Decanoic Acid	0	CH_3CN	1 hour	2.7	0.62
Decanoic Acid	2	CH_3CN	1 hour	19.2	0.38
Decanoic Acid	5	CH_3CN	1 hour	38.2	0.40
Decanoic Acid	10	CH_3CN	1 hour	55.7	0.46
Ac-D,L-Leu-OH	0	CH_3CN	1 hour	2.7	0.62
Ac-D,L-Leu-OH	2	CH_3CN	1 hour	25.1	0.55
Ac-D,L-Leu-OH	5	CH_3CN	1 hour	31.7	0.59
Ac-D,L-Leu-OH	10	CH_3CN	1 hour	44.0	0.64
Ac-D,L-Leu-OH	2	CH_3CN	3 days	86.0	0.61
Ac-D,L-Leu-OH	5	CH_3CN	3 days	69.3	0.70
Ac-D,L-Leu-OH	10	CH_3CN	3 days	68.0	0.73
Ac-L-Leu-OH	0	CH_3CN	1 hour	2.6	0.53
Ac-L-Leu-OH	2	CH_3CN	1 hour	18.4	0.52
Ac-L-Leu-OH	5	CH_3CN	1 hour	32.9	0.57
Ac-L-Leu-OH	10	CH_3CN	1 hour	45.5	0.62

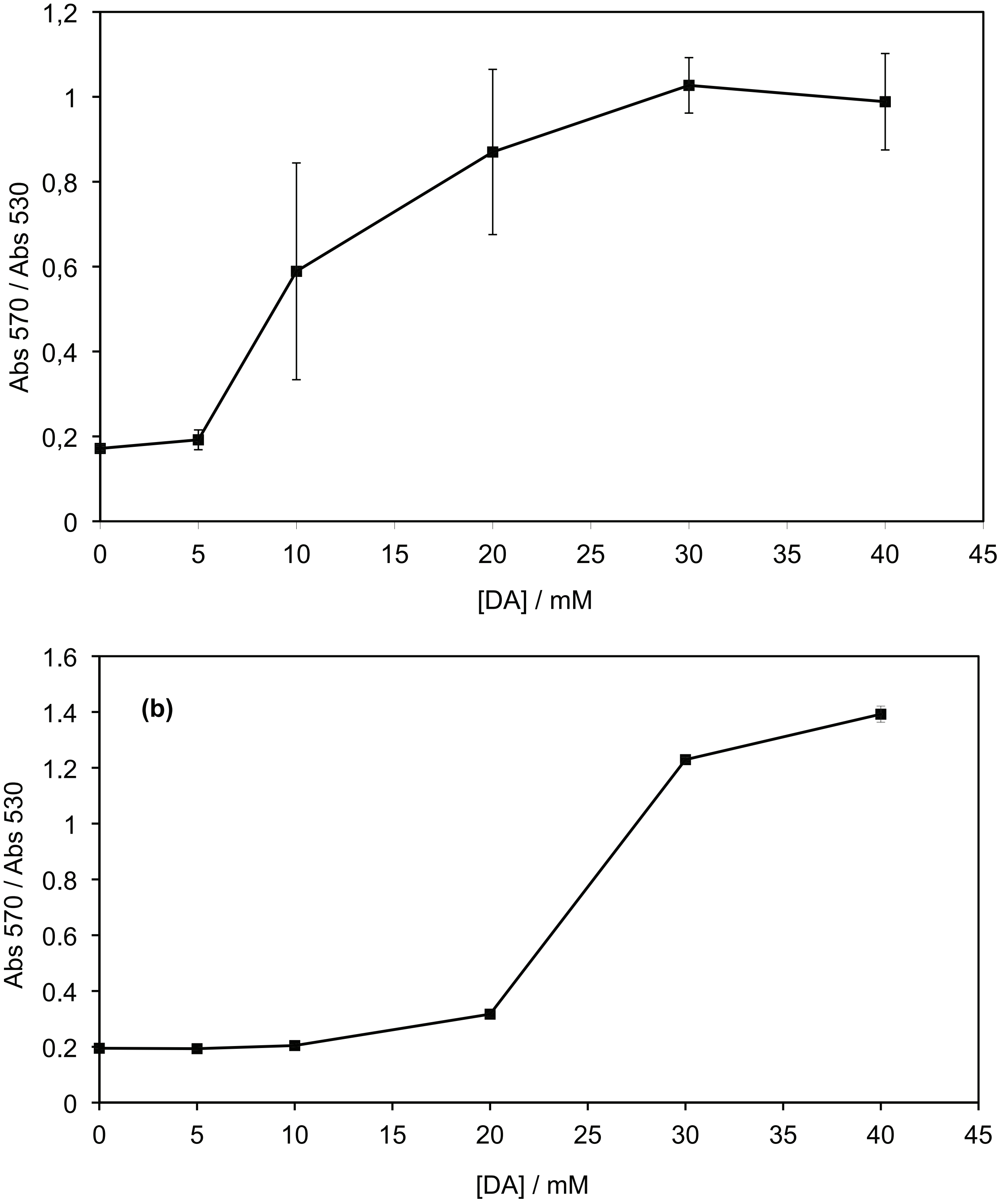
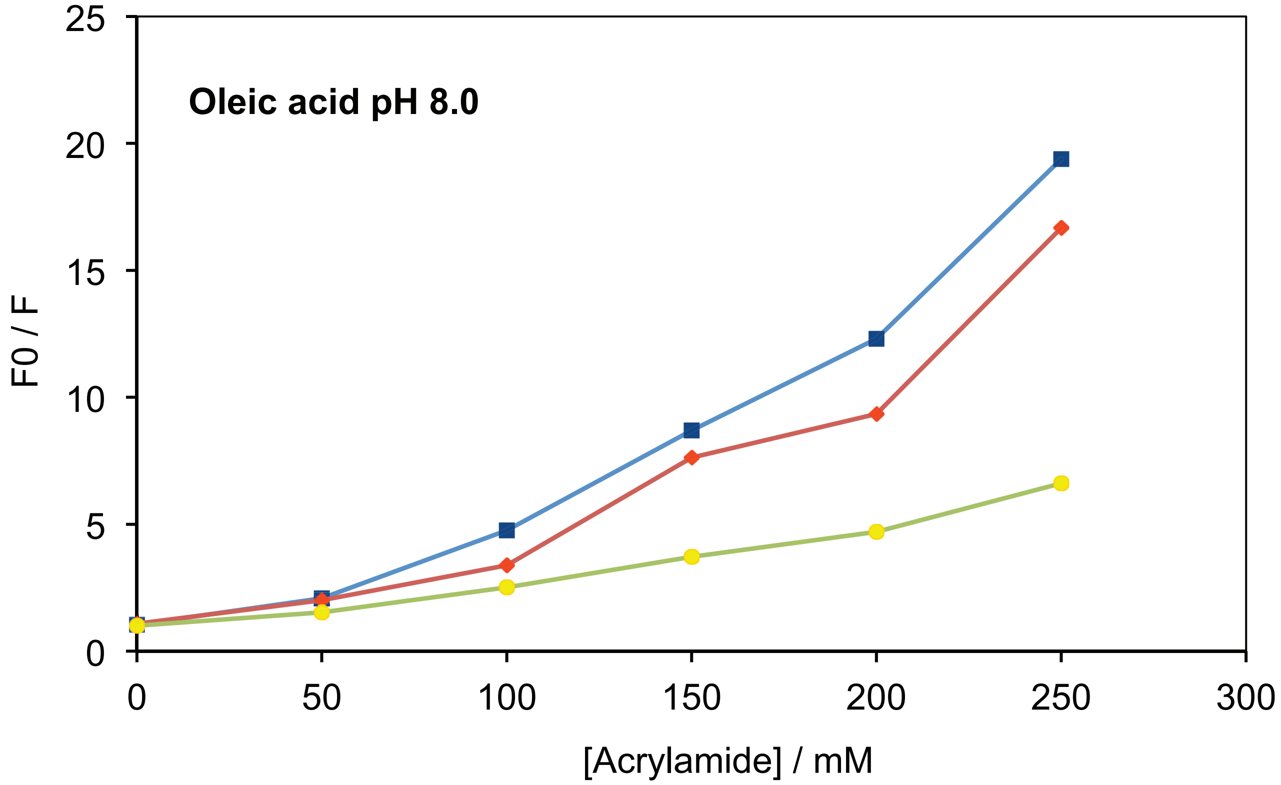


Fig. S2 Measurement (Merocyanine 570 assay) of the CVC of decanoic acid (DA) in **(a)** 100 mM MES buffer at pH 6.5; and **(b)** 100 mM MOPS buffer at pH 7.2; both in the presence of 5 mM H-Leu-NH_2. The large error bars in panel **(a)** reflect the instability of the dispersion at pH values departing from the effective pKa of decanoic acid. Less reproducible results are obtained in that pH range (or lower) due to the formation of inhomogeneous droplets. A similar protocol was followed to determine the CVC of dodecanoic acid (DoA) and oleic acid (OA).



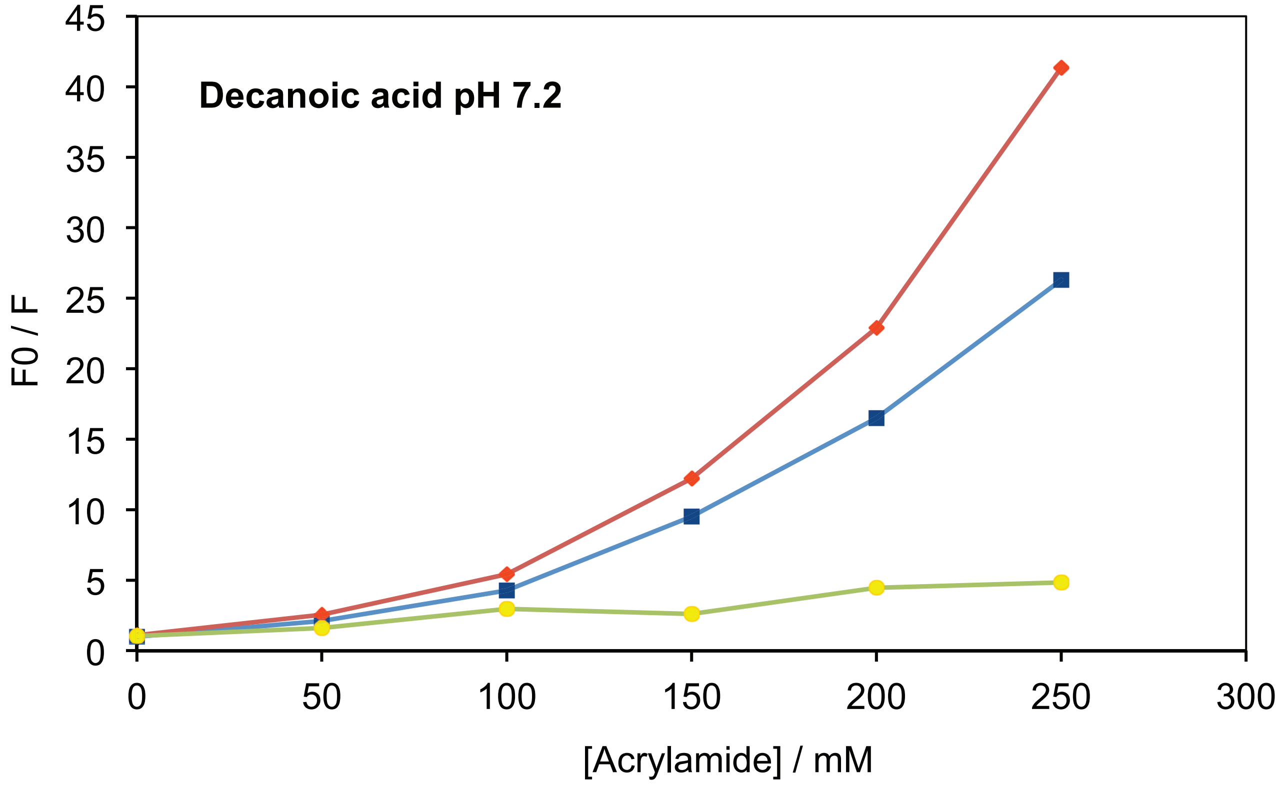


Fig. S3 Binding of Ac-L-Tyr(Me)-L-Leu-NH$_2$ (1 µM overall concentration) to the vesicles in 100 mM MOPS buffer solutions to which increasing amounts of acrylamide were added; control experiment without fatty acid (blue squares); fatty acid concentration (0.05 mM for oleic acid and 10 mM for decanoic acid) below the CVC (red diamonds); fatty acid concentration (1 mM for oleic acid and 40 mM for decanoic acid) above the CVC (yellow filled circles). Lower slope values show a higher protection of the membrane against quenching by acrylamide.

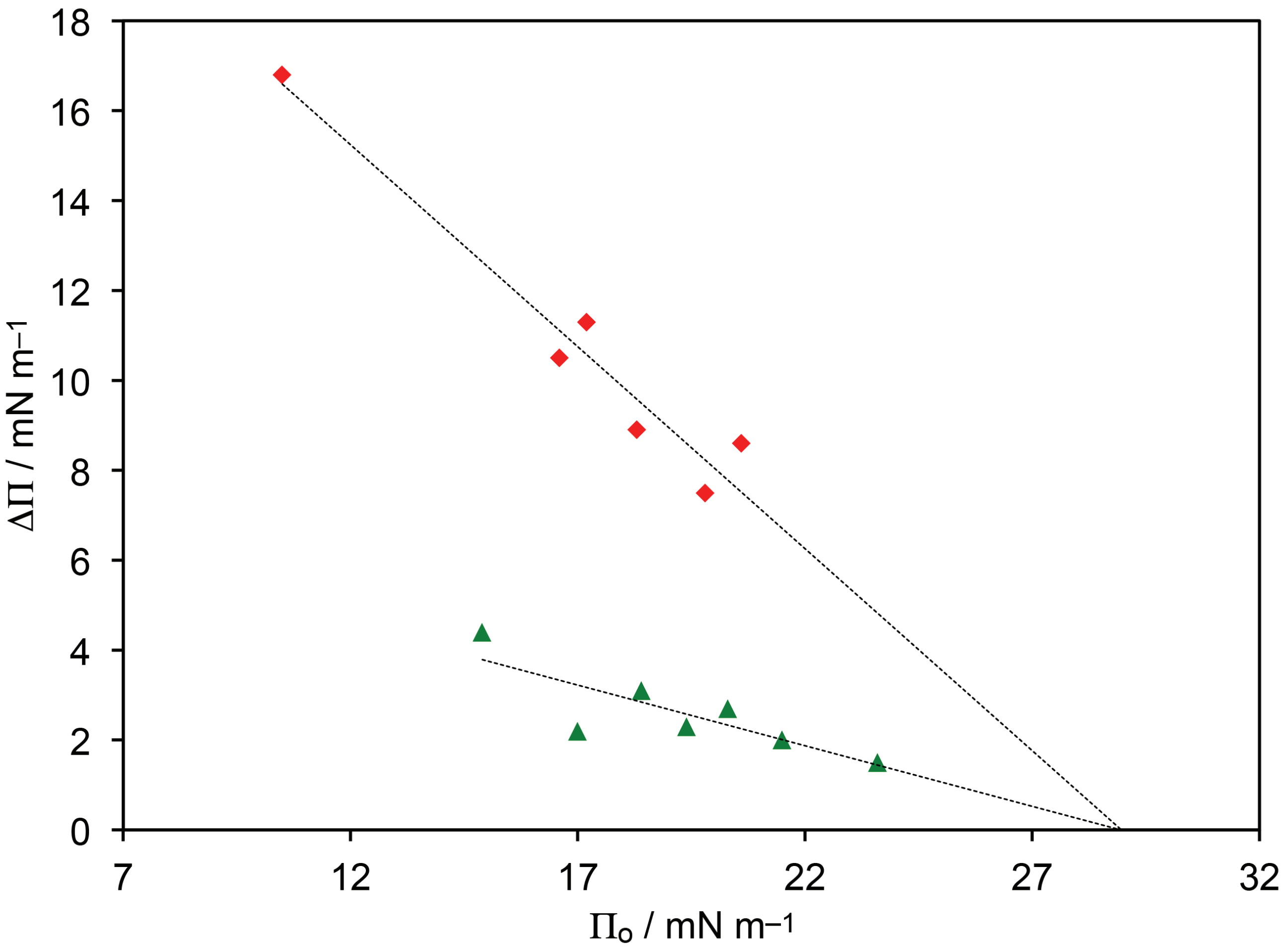


Fig. S4 Interaction of the dipeptide Ac-L-Tyr(Me)-L-Leu-NH_2 with decanoic acid monolayers at different initial surface pressures (π_0). DA monolayers were prepared with different initial surface pressures. The dipeptide was injected into the aqueous subphase and the resulting increase in surface pressure ($\Delta\pi$) was recorded. This increment in surface pressure is due to the adsorption of the dipeptide to the fatty acid monolayer. The experiment was carried out at pH 6.5 (red diamonds) and pH 7.2 (green triangles). The intercept of the regression lines with the abscissa axis yields the critical pressure (π_c), *i. e.* the initial surface pressure at which the dipeptide is no longer able to adsorb to the monolayer. Notice that the critical pressure is the same for both pH values. This indicates that the interfacial properties of the monolayer do not change with the pH.

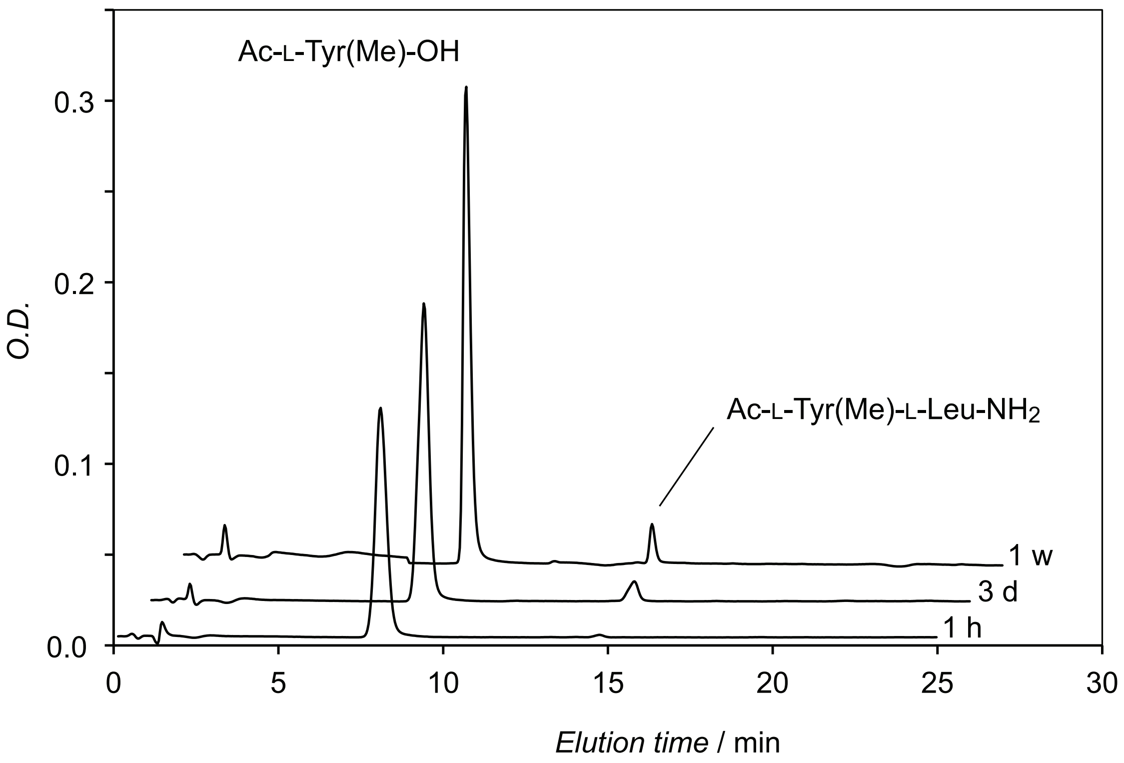


Fig. S5 HPLC analysis of the reaction media obtained by reaction of 2 mM Ac-Leu oxazolone with 5 mM H-L-Leu-NH_2 in the presence of 1 mM Ac-L-Tyr(Me)-OH in CH_3CN; analysis of sample withdrawn after 1h, 3 days and 1 week. The detection wavelength value of 273 nm allows monitoring the products of Ac-L-Tyr(Me)-OH bearing the methylated phenol moiety separately from other species.