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Supplementary information

Fragment-based approach to study fungicide-biomimetic membrane interactions

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Experimental details

1. High-resolution mass spectra of the synthesized molecules
2. Determination of apparent distribution constants
3. High performance liquid chromatography
4. Micro differential scanning calorimetry
5. Open-tubular capillary electrochromatography
6. Nanoplasmonic sensing
7. Quartz crystal microbalance

1. High-resolution mass spectra of the synthesized molecules

Stock solutions of the corresponding hydrochloride salts in methanol were prepared at a concentration of 20 µg/mL and then further diluted to a concentration of 1 µg/mL for mass spectrometric (MS) analysis. The Q Exactive HF Orbitrap mass spectrometer (Thermo Fisher Scientific (Bremen) GmbH, Germany) was operated in positive ESI mode for all measurements. Data collection was performed in full mass scan mode at a resolution of 120 000 covering a mass range ± 100 mass units relative to the expected m/z signals. The automatic

gain control was set to $1 \cdot 10^6$ and the maximum injection time to 100 ms. The spray voltage was set at 3.5 kV, the capillary temperature at 320 °C, with the S-lens RF, sheath gas and Aux gas levels being set at 50, 8 and 1 arbitrary units, respectively. All samples were infused at a flow rate of 5 µL/min via an electronically controlled syringe pump. Figures S1-S5 shows the data on the compounds.

2. Determination of apparent distribution constants

For HPLC analysis, the phases were settled by centrifuging the vials for 2 min at 4000 rpm, followed by isocratic reversed-phase (RP) HPLC analysis. The mobile phase composition (acetonitrile/water, 0.05% formic acid) for the individual compounds was adjusted so to ensure fast elution and acceptable peak shapes, with peaks being recorded by UV absorption detection at 210 and 230 nm. Selective injection of 1.0 µL aliquots of the individual phases was accomplished by adjusting the injector needle position (1-octanol: 10 mm injection height; buffer phase: 3 mm injection height) appropriately. The integrated peak areas found for the 1-octanol and buffer phase (average of two injections each) were divided (area 1-octanol/area buffer) to obtain the experimentally determined apparent log $D_{pH7.4}$ values.

3. High performance liquid chromatography

The eluents were pump-mixed to appropriate isocratic compositions from pre-prepared mobile phases (MP A and MP B), with MP A consisting of ACN-water-formic acid 50:1000:0.5 (v/v/v) and MP B of ACN-water-formic acid 50:50:0.5 (v/v/v), respectively. All chromatographic experiments were conducted using a 100 x 3 mm i.d. Luna C18(2) (5 µm, 100 Å) column, equipped with a pre-column, at a flow rate of 0.50 mL/min. Signals were detected at 210, 230, 254 and 280 nm, respectively, with the reference wavelength set to 480 nm. The column temperature was kept at 25° C. If not stated otherwise, the sample concentrations were 1.0 mg/mL, and the injection volume was 1.0 µL. Data acquisition and analysis were carried out with the Agilent ChemStation (Rev. B.01.03-SR2) software package. For LC-UV-MS measurements, the Agilent 1260 Series HPLC instrument was hyphenated via a PEEK transfer line with a Bruker Esquire 3000plus ion trap mass spectrometer. For this purpose, th esquireControl Version 6.1 and esquireDataAnalysis Version 3.4 software packages, respectively.e post-column flow was split using a zero-volume T-union, with one of the split streams being delivered to the diode array detector, while the other being infused into the electrospray ion source of mass spectrometer. The mass spectrometer was operated in positive ion mode, with the capillary and endplate offset voltages set to -4000 and -500 V; the

nebulizer pressure to 50 psi; the dry gas flow rate to 12.0 L/min; and the source temperature to 360° C. Data acquisition and analysis were conducted using the esquireControl Version 6.1 and esquireDataAnalysis Version 3.4 software packages, respectively.

4. Micro differential scanning calorimetry

The sample cell of the calorimeter was filled with 250 µL of lipid or lipid-analytes suspension, whereas the reference cell was filled with PBS buffer. At least three consecutive heating and cooling cycles from 0 to 90 °C were performed with a scan rate of 1 °C/min. The repeated heating (and cooling) thermograms were essentially identical in shape. Thermograms shown in the figures are those obtained with the first heating scans.

5. Open-tubular capillary electrochromatography

Before the first use, the capillaries were flushed for 20 min with 0.1 M NaOH, then for 30 min with ultrapure water and as a final step, for 5 min with HEPES. The samples were first run using HEPES as the background electrolyte. All sample runs were repeated three times, and in between each run, the capillary was rinsed for 1 min with HEPES. The samples were injected hydrodynamically (15 mbar, 20 s). Operation voltage was 30 kV and temperature 25 °C. UV spectra of the runs were recorded at wavelengths 190-400 nm with 2 nm steps and the spectra of the analytes were saved in the UV spectrum library. Electropherograms for data analysis were extracted for the wavelengths 200, 214, 254 and 280 nm.

For liposome coating, the capillary was preconditioned by rinsing the capillary at high pressure (930 mbar) with the following solutions: first for 10 min with 0.5 M nitric acid; then for 15 min with ultrapure water; and finally for 1 min with 3 mM calcium-HEPES solution. The preconditioning was followed by the coating step: first, the capillary was rinsed for 10 min with liposome dispersion (100 mol% POPC, 90:10 mol% POPC/DOPE, or 80:10:10 mol% POPC/DOPE/Chol), and then left to stand filled with the liposome solution for 15 min. Finally, the capillary was rinsed for 2 min with 10 mM HEPES. All sample runs were repeated three times with same operational parameters as with the uncoated capillary. Before each sample injection, the capillary was rinsed for 0.5 min with the liposome solutions and for 1 min with the HEPES buffer. All sample runs were carried out in triplicate.

The strength of the liposome-analyte interactions in OT-CEC can be described by the retention factor k. For uncharged analytes the retention factor can be calculated as:

$$k = \frac{t_m - t_{eo}}{t_{eo}} \quad (1)$$

where tm and teo are migration times of the analyte and the electroosmotic flow marker, respectively. For charged analytes, the retention factor k’ can be utilised:

$$k^{'} = \frac{t_m\left(1 + k_e^{'}\right) - t_{eo}}{t_{eo}} \quad (2)$$

where k_e' is the velocity factor, described as $k_e' = \mu_{ep}/\mu_{eo}$, where μ_{ep} is the electrophoretic mobility of the analyte determined in an uncoated capillary, and μeo is the electrophoretic mobility of the electroosmotic flow in the coated capillary [1].

For the calculation of the distribution coefficients, K_D, equation (3) was used

$$K_D = k/\phi \quad (3)$$

where k (or k’) is the retention factor of the analyte and ϕ is the phase ratio, e.g., the volume ratio of the lipid and the aqueous phase. For calculating the phase ratio ϕ for the OT-CEC system the following equation was used [2]:

$$\phi = \frac{2 \cdot h}{r} - \frac{h^2}{r^2} \quad (4)$$

where h is the thickness of the coating layer estimated to 4 nm (see reference 3) and r is the radius of the capillary (25 μm).

6. Nanoplasmonic sensing

Before each measurement, the sensors were sonicated for 5 minutes in ethanol. They were then pretreated with oxygen plasma using an UV-ozone cleaner (UVC-1014 NanoBioAnalytics, Berlin, Germany). After each measurement the sensors were rinsed with ethanol and water. Additionally, between the use of different liposome compositions, the measurement cell and tubing were rinsed with an aqueous 1 wt% sodium dodecyl sulphate solution for 5 minutes.

The solutions used for the preconditioning (0.1 M NaOH, Milli Q water, and 10 mM HEPES buffer with or without Ca^{2+}) were degassed for 30 minutes in bath sonication before each

experiment to remove air bubbles. Experiments were performed with a continuous flow of 100 μL/min and at a constant temperature of 25 °C. Measurement data was acquired by the Insplorion S2 software InControl v.1.0.2.

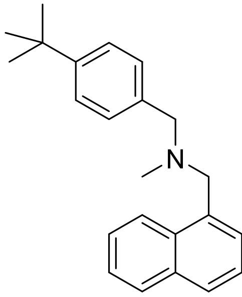
The measurement cell was subsequently rinsed with Milli-Q water, NaOH solution, and HEPES buffer for 5 min each. Finally, the sensor surface was flushed with Ca-HEPES buffer for 10 min to obtain a stable peak shift ($\Delta\lambda$) signal. The liposomes were then introduced into the measurement cell until a new stable $\Delta\lambda$ baseline was reached, indicating the saturation of the sensor surface. For the removal of non-adsorbed vesicles from the surface, the sensor was rinsed again with Ca-HEPES buffer followed by Milli-Q water for 3 min each.

7. Quartz crystal microbalance

Prior to each run, the sensor was first boiled for 5 min in a mixture of $NH_4OH/H2O_2/H_2O$ in volume ratio 1:1:5 to activate the SiO_2 surface, then rinsed with deionized water, and finally dried with a gentle stream of air. After mounting the sensor into the flow cell, it was rinsed with Milli Q water at 100 μL/min until a stable frequency was reached. The liposomes were adsorbed after preconditioning of the sensor by a sequential rinse for 1 min with water, 3 min with 10 mM HEPES buffer, and 2 min with 10 mM HEPES buffer with 5 mM $CaCl_2$. Following a 2 min rinse with liposomes, the flow rate was lowered to 10 μL/min and liposomes were for additional allowed to flow passed the sensor for 32 minutes. The valve was switched to rinsing with Ca-HEPES and the flow rate was again increased to 100 μL/min for 5 minutes. Finally, the system was switched to rinsing with Milli Q water.

Figure captions

Figure S1. MALDI-TOF spectrum of butenafine (compound B).
Figure S2. MALDI-TOF spectrum of fragment F1 (compound F1).
Figure S3. MALDI-TOF spectrum of fragment F2 (compound F2).
Figure S4. MALDI-TOF spectrum of fragment F3 (compound F3).
Figure S5. MALDI-TOF spectrum of control compound C (compound C).
Figure S6. Representative DSC heating thermograms of lipid vesicles.



Chemical Formula: $C_{23}H_{27}N$
Exact Mass: 317.21

Compound B

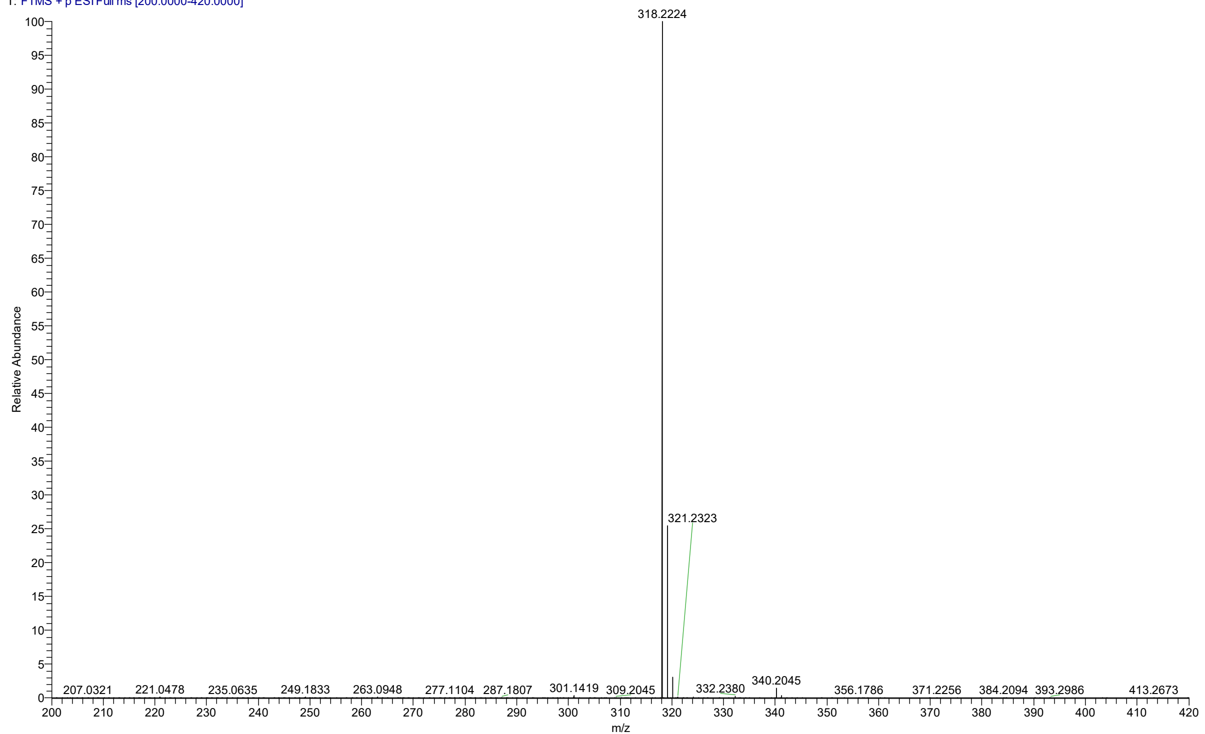
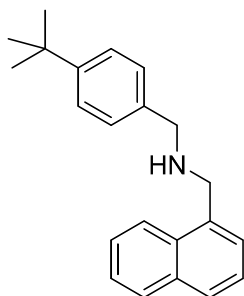


Figure S1. MALDI-TOF spectrum of butenafine (compound B). Detected Ion: $[M-H]^+$; Mass theory: 318.2216 amu; Mass exp.: 318.2224 amu; Dm = -2.51 ppm



Chemical Formula: $C_{22}H_{25}N$
Exact Mass: 303.20

Fragment 1

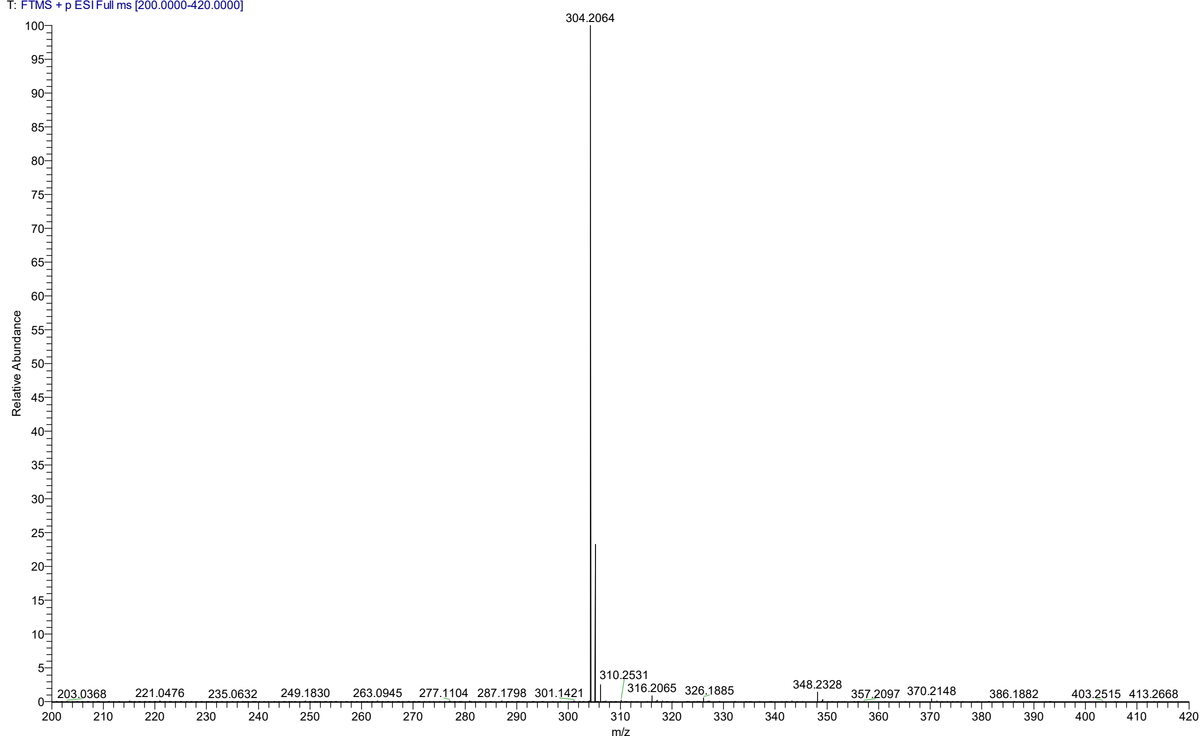
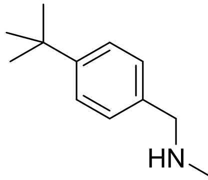


Figure S2. MALDI-TOF spectrum of fragment F1 (compound F1). Detected Ion: $[M-H]^{+}$; Mass theory: 304.2060 amu; Mass exp.: 304.2064 amu; Dm = -1.32 ppm



Chemical Formula: $C_{12}H_{19}N$
Exact Mass: 177.15

Fragment 2

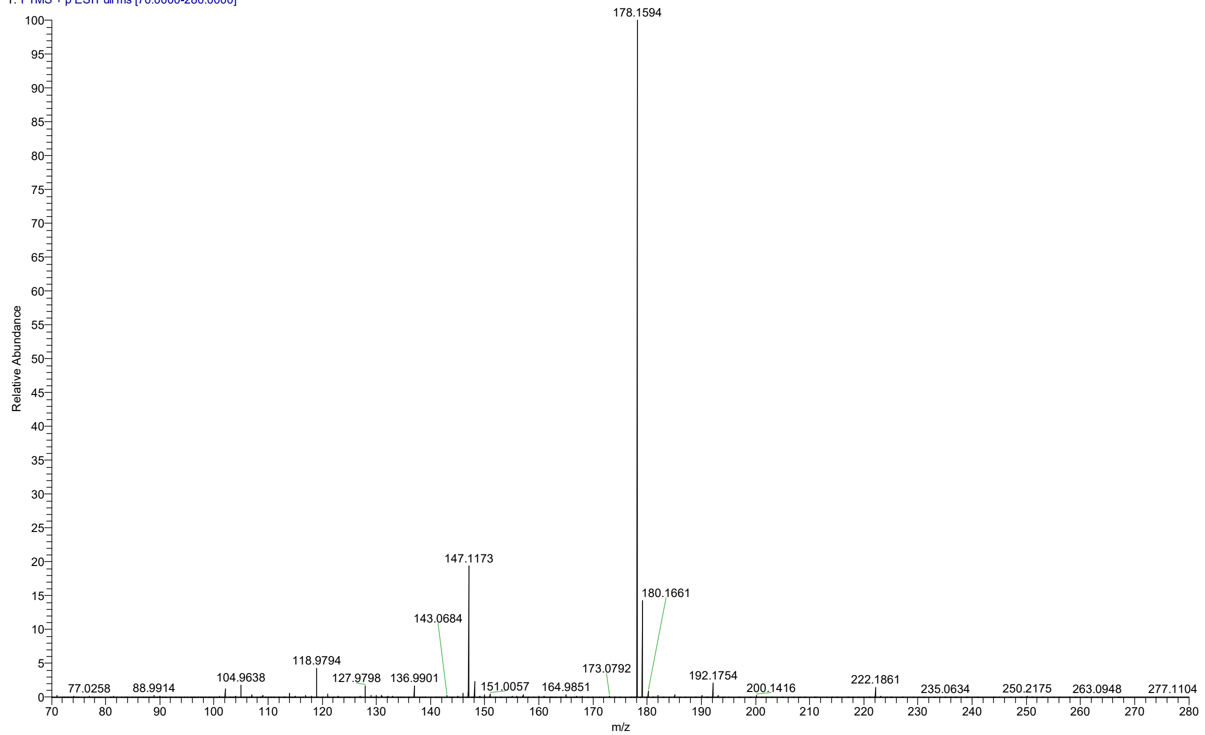
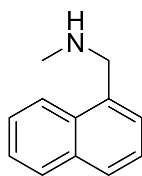


Figure S3. MALDI-TOF spectrum of fragment F2 (compound F2). Detected Ion: $[M-H]^{+}$; Mass theory: 178.1590 amu; Mass exp.: 178.1594 amu; Dm = -2.25 ppm



Chemical Formula: $C_{12}H_{13}N$
Exact Mass: 171.10

Fragment 3

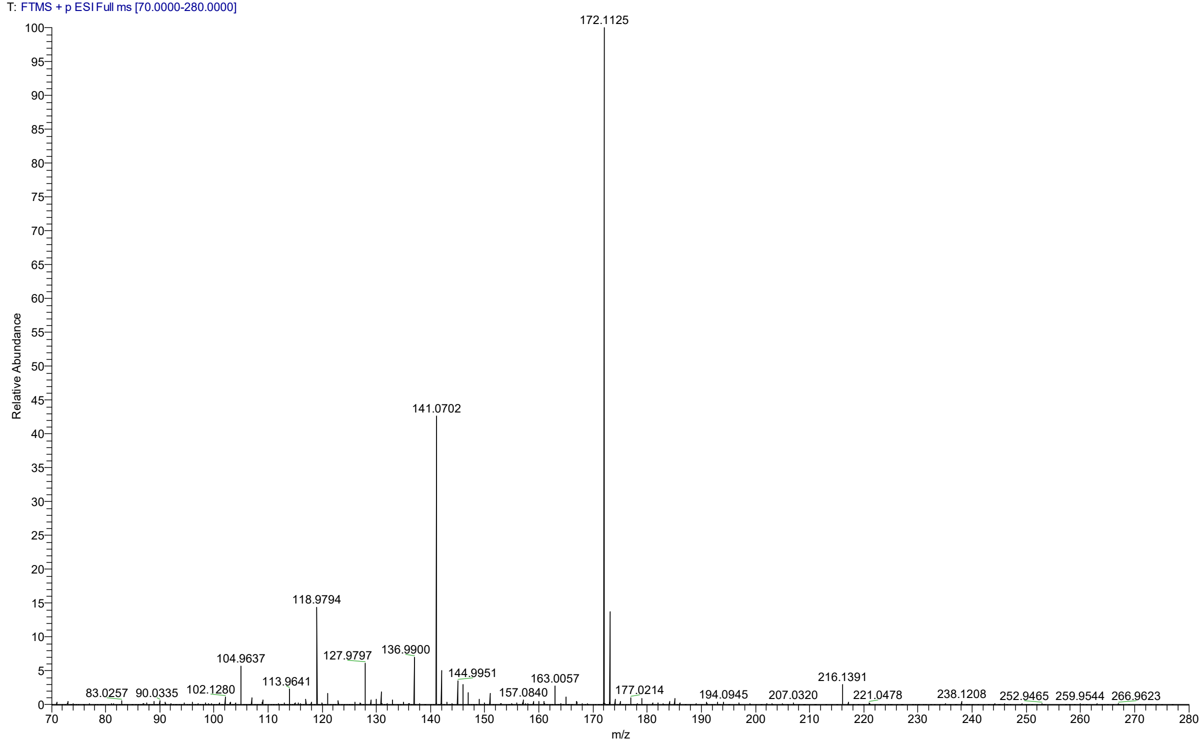
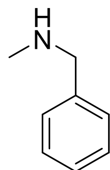


Figure S4. MALDI-TOF spectrum of fragment F3 (compound F3). Detected Ion: $[M-H]^+$; Mass theory: 172.1121 amu; Mass exp.: 172.1125 amu; Dm = -2.13 ppm



Chemical Formula: $C_8H_{11}N$
Exact Mass: 121.09

Compound C

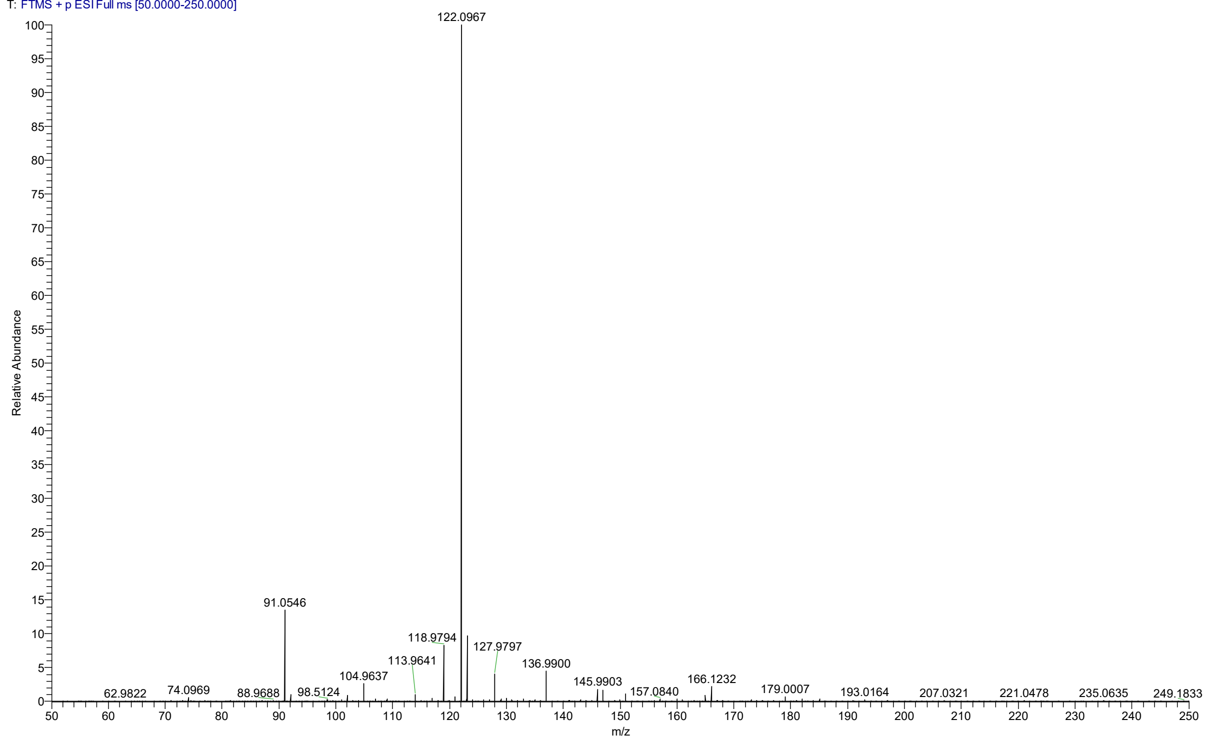


Figure S5. MALDI-TOF spectrum of control compound C (compound C). Detected Ion: [M-H]$^{+}$; Mass theory: 122.0964 amu; Mass exp.: 122.0967 amu; Dm = -4.39 ppm

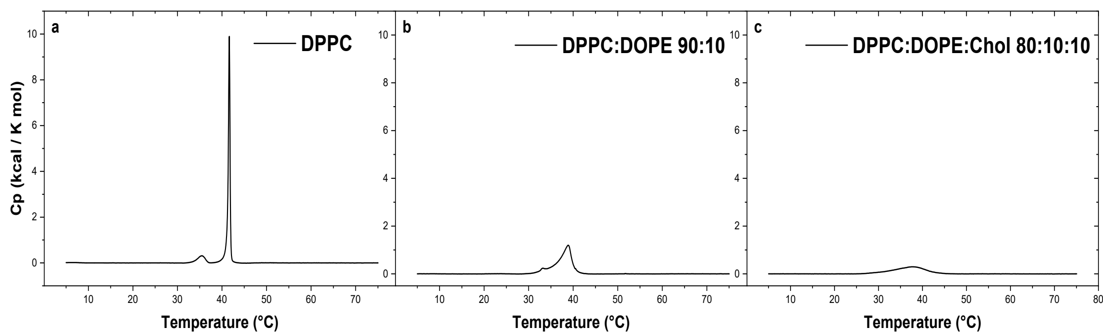


Figure S6. Representative DSC heating thermograms of lipid vesicles (a), DPPC; (b) DPPC:DOPE (90:10 mol%) and (c) DPPC:DOPE:Chol (80:10:10, mol%). The samples were heated at a rate of 1°C/min. Thermograms shown are the first heating scans.

References:

1. K. Lipponen, S. K. Wiedmer and M.-L. Riekkola, *Journal of Chromatography Open*, 2021, **1**, 100020.
2. F. Duša, J. Witos, E. Karjalainen, T. Viitala, H. Tenhu and S. K. Wiedmer, *Electrophoresis*, 2016, **37**, 363-371.
3. D. Regan, J. Williams, P. Borri and W. Langbein, *Langmuir*, 2019, **35**, 13805-13814.

[Refs 1-3 correspond to refs 18-20 in the main manuscript].