Supplementary Information

Chemicals

The phospholipids 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine (DPPE), 1,2-dipalmitoyl-*sn*-glycero-3-phospho-(1'-*rac*-glycerol) sodium salt (DPPG), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine (POPE), and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-(1'-*rac*-glycerol) sodium salt (POPG) were purchased from Avanti Polar Lipids (Alabaster, USA). Magnesium perchlorate, magnesium sulphate, magnesium chloride, the corresponding sodium salts and the HEPES buffer were purchased from Merck (Darmstadt, Germany). The fluorescent probes *N*-(lissamine rhodamine B sulfonyl)-1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine triethylammonium salt (N-Rh-DHPE), ATTO-647, and Laurdan (6-dodecanoyl-N,N-dimethyl-2-naphthylamine) were purchased from Molecular Probes (Invitrogen, California, USA), ATTO-TEC (Siegen, Germany) and Merck, respectively. Phopholipase A2 from honey bee venom (bvPLA2) was purchased from Merck and its fluorogenic substrate 1-O-(6-Dabcyl-aminohexanoyl)-2-O-(12-(5-BODIPY®-pentanoyl)aminododecanoyl)-*sn*-glyceryl phosphatidylcholine (DBPC) was purchased from MoBiTec (Goettingen, Germany).

Methods

Vesicles preparation

Vesicles were prepared by weighing appropriate amounts of lipid stock solutions and evaporating the organic solvent under a stream of nitrogen [42] to obtain a suspension of multilamellar vesicles (MLVs). Large Unilamellar Vesicles (LUVs) of 100 nm size were obtained by extruding MLVs at least 21 times through two stacked polycarbonate filters and a permeable membrane with 100 nm pores (Nuclepore, Pleasanton, USA). The whole procedure was carried out using an extruder (Avanti Polar Lipids) filled with two 1.0 mL Hamilton syringes (Hamilton, Reno, USA). Vesicles size was confirmed by means of dynamic light scattering (DLS) measurements, which showed a mean hydrodynamic radius consistent with the formation of LUVs. Giant Unilamellar Vesicles (GUVs) were prepared with the electroformation method using a 1 mg mL^{-1} lipid solution. For the electroformation, a constant frequency of 500 Hz and an alternating electric field of 0.14 V (5 min), 1.25 V (20 min), and 3.5 V (90 min) was applied at a temperature of 45 °C. GUVs were used for fluorescent microscopy measurements, LUVs were used for enzyme kinetics measurements while MLVs were used in all the other cases. DPPE/DPPG in a 8/2 ratio were used for the DSC measurements, while POPE/POPG at a 8/2 ratio was used in all the other cases.

Differential scanning calorimetry (DSC)

Heat capacity curves of a 500 μM DPPE/DPPG suspension were recorded in buffer and in the presence of magnesium chloride, magnesium sulphate, magnesium perchlorate, and the corresponding sodium salts at concentrations of 0.25 M, 0.5 M, and 1 M using a high sensitivity Nano DSC (TA Instruments, New Castle, DE, USA) equipped with 300 μL twin gold capillary cells, pressurized to 3 atm. Every sample was scanned from 25 to 70 °C at least four times, using a scanning rate of 1 °C/min.

Fluorescence spectroscopy

Fluorescence spectroscopy measurements were carried out on a K2 multifrequency phase modulation fluorometer (ISS Inc., Champaign, IL, USA), equipped with a variable temperature and a high-pressure optical pressure cell equipped with sapphire windows allowing pressure dependent measurements up to about 2 kbar. The Laurdan probe was embedded into POPE/POPG vesicles in a 1:30 probe:lipid ratio. In order to monitor changes in lipid order parameter/hydration level and thus the lipid phase of the membrane, the generalized polarization parameter *GP* was used. The *GP* value is defined as $GP = (I_{440} - I_{490})/(I_{440}+I_{490})$ where I_{440} and I_{490} are the intensities at 440 nm and 490 nm, respectively of the Laurdan emission spectrum. A low *GP* value is associated with a more hydrated fluid state of the lipid membrane, while a high *GP* value (around 0.5) is associated with a less hydrated ordered (gel) state of the lipid bilayer. Laurdan emission spectra were recorded in the 390-580 nm range with an excitation wavelength of 340 nm and both excitation and emission slits set to 8 nm. A total lipid concentration of 50 μM was used in buffer and in the presence of 0.25, 0.5, and 1 M salt concentration.

Enzyme kinetics

In the enzyme kinetics experiment, the fluorogenic analogue of the PLA2 substrate PC, DBPC, was used. The fluorescent probe BODIPY in the *sn*-1 position is quenched by the 6-Dabcyl-aminohexanoyl (DBPC) in position *sn*-2. Once the PLA2 hydrolyzes the *sn*-2 phosphodiester bond, a strong increase in BODIPY fluorescence is observed due to the loss of spatial correlation between the two fluorophores. The measurements were performed on the same K2 multifrequency phase modulation fluorometer reported above. An excitation wavelength of 488 nm was used while the kinetics was measured at the emission wavelength of 518 nm. Both emission and excitation slits were set to 8 nm, the time-step kinetics to 1 s, and the temperature was set to 35 °C. The pressure-dependent kinetic measurements were performed using an enzyme concentration of 0.01 μM, with the lipid concentration fixed at 150 μM, monitoring the fluorescence changes for a time period of 1800 s.

Confocal microscopy

For confocal fluorescence microscopy imaging, the laser combiner Oxxius Simply Light, 4Cc-CSB-130 (Lannion, France) in combination with a mercury-vapor lamp (Hg 100 W, Nikon, Tokyo, Japan) and the objective lens type CFI Plan Apochromat Lambda 100x Oil, NA 1.45, WD 0.13 (Nikon, Tokyo, Japan) were used. GUVs were grown in a 100 mM NaCl solution with 10 mM HEPES buffer at pH 7.4. Salt solutions were added after vesicle growth with a syringe. Images were postprocessed and analysed with the open source ImageJ software to obtain average size and distribution.

Small-angle X-ray scattering (SAXS)

SAXS measurements were performed on a SAXSess mc^2 instrument from Anton-Paar (Graz, Austria) with a temperature control unit (TCS Control Unit, Anton-Paar, Graz, Austria). The X-ray radiation was generated with a copper X-ray tube, and it was directed onto a quartz capillary cell. The scattering was detected in an evacuated chamber to reduce air scattering for every sample within 30 min via imaging plates in a temperature range of 10-40 °C. The capillary cell was filled with 10 μL of the 10% w/w lipid suspension.

In a typical diffractogram, the appearance of Bragg-peaks in the elastic scattering curve, I(Q) allows the determination of the lamellar repeating distance and the lipid phase. Q is the modulus of the wave vector transfer, given by $Q = (4\pi/\lambda)\sin\theta$, with 2θ being the scattering angle and λ the wavelength of the X-rays.[25] The type of phase can be distinguished by the characteristic SAXS peak ratios in *Q*-

space (Q_1:Q_2:Q_3: ...). For the lamellar lipid phase, they are equidistant (1:2:3: ...). According to Bragg's equation, the lattice parameter for the lamellar phase is given by $d_{lam}= 2\pi/Q_1$. The lamellar repeat distance, d_{lam}, includes the thickness of the MLV's interlamellar water layer, d_w, and the thickness of the lipid bilayer, d_l, i.e., $d_{lam} = d_w + d_l$

Circular dichroism spectroscopy

Circular dichroism (CD) measurements were performed on a Jasco J-715 spectropolarimeter (Jasco Analytical Instruments, Tokyo, Japan). The spectra were recorded at 35 °C in the 260-200 nm range with 0.5 nm resolution, 4 nm bandwidth, and 2 s of integration time. The spectra were averaged over five accumulations. The protein concentration used was 3 µM.

Additional figures

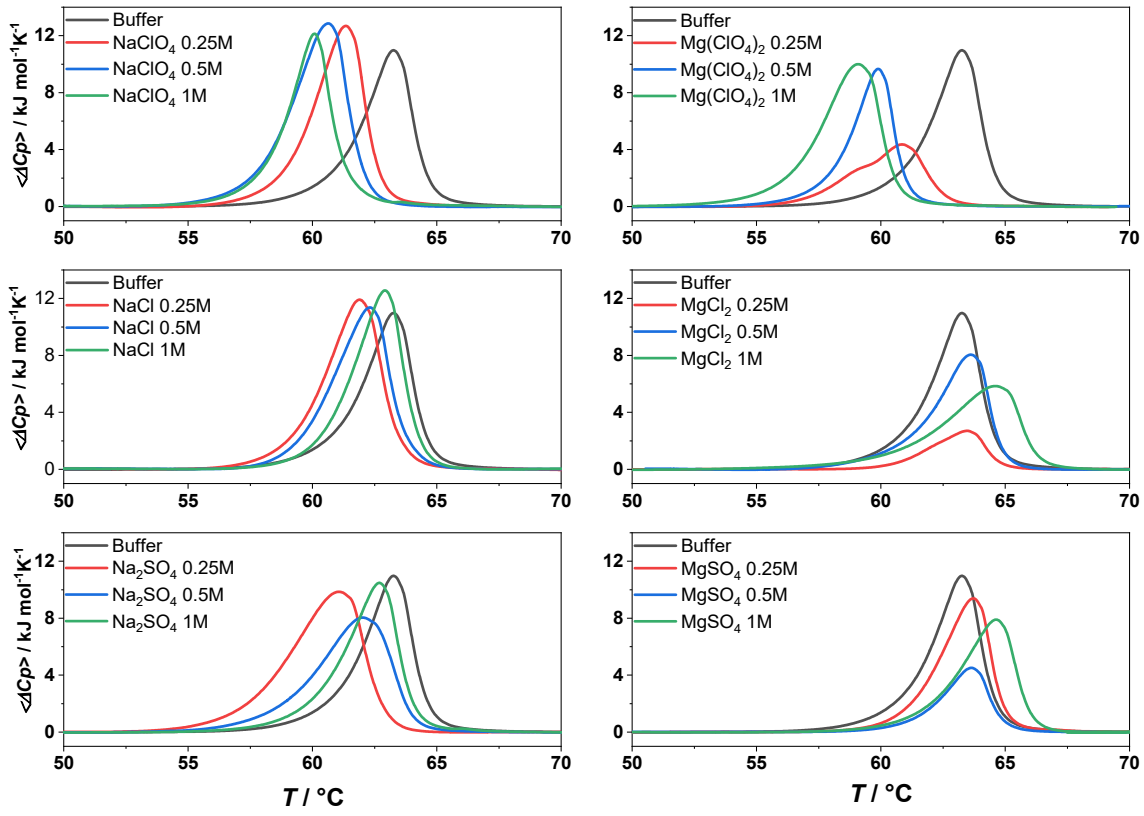

Figure S1 DSC traces of DPPE/DPPG 8/2 multilamellar vesicles in the presence of different Martian relevant salts at 0.25, 0.5, and 1M concentration, as indicated in the insets. All the experiments were performed in 10 mM HEPES buffer, pH 7.4.

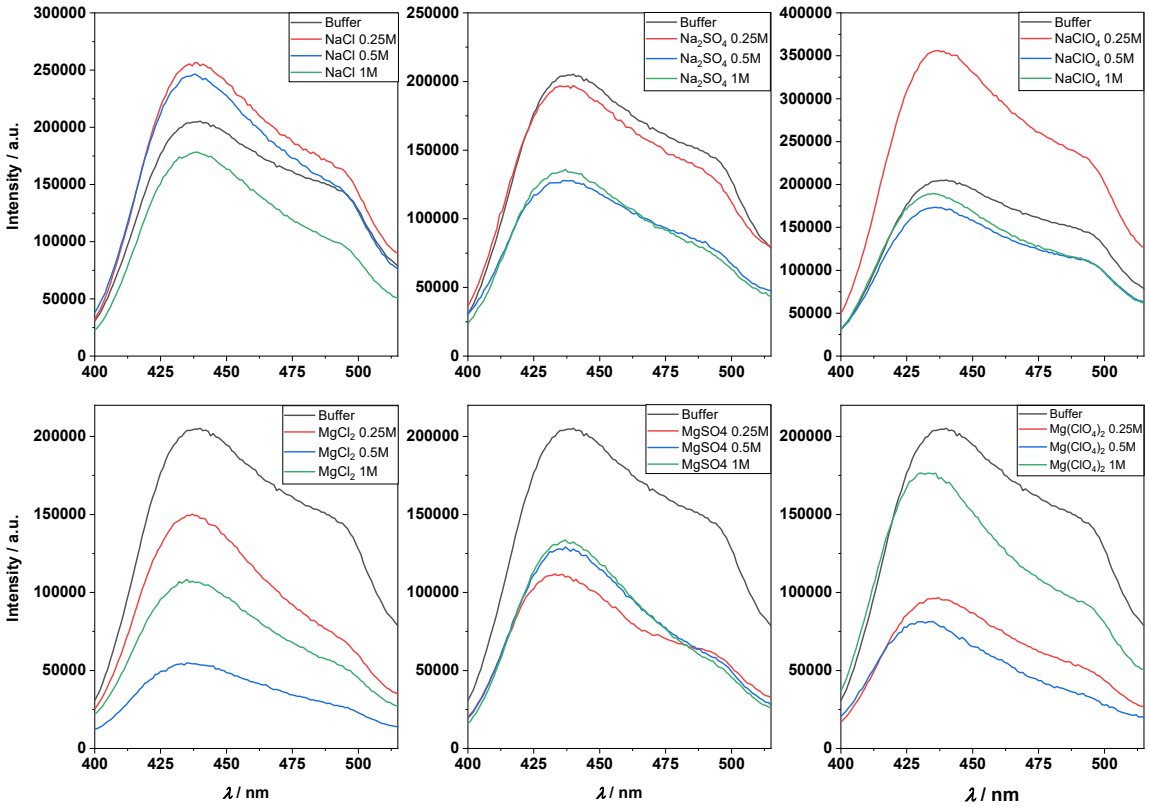

Figure S2 Laurdan emission spectra at 1 bar in the presence of 0.25, 0.5, and 1 M of different Martian salts, as indicated in the insets. All the experiments were performed in 10 mM HEPES buffer, pH 7.4, at the temperature of 35 °C.

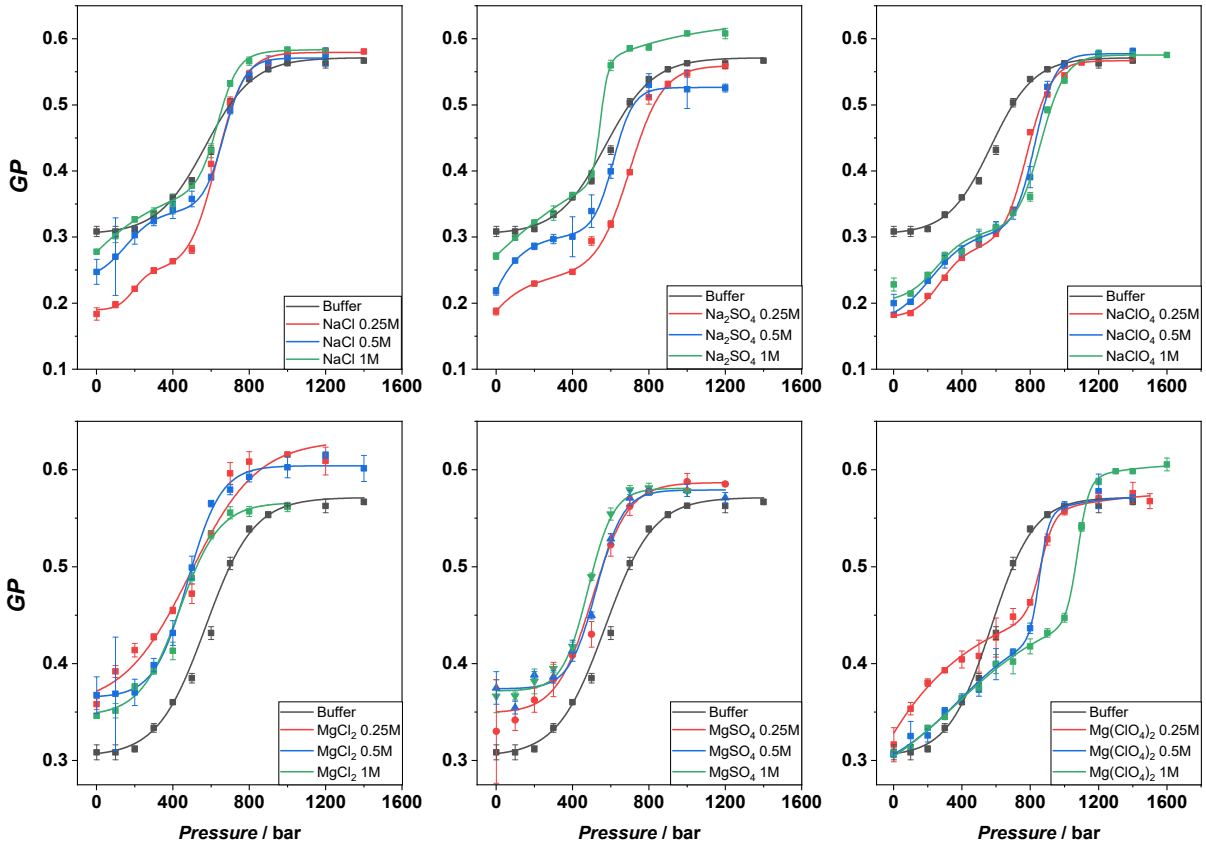

Figure S3 Pressure dependent *GP* curves of Laurdan embedded in POPE/POPG 8/2 vesicles in the presence of 0.25, 0.5, and 1 M of different Martian salts, as indicated in the insets. All the experiments were performed in 10 mM HEPES buffer, pH 7.4, at the temperature of 35 °C.

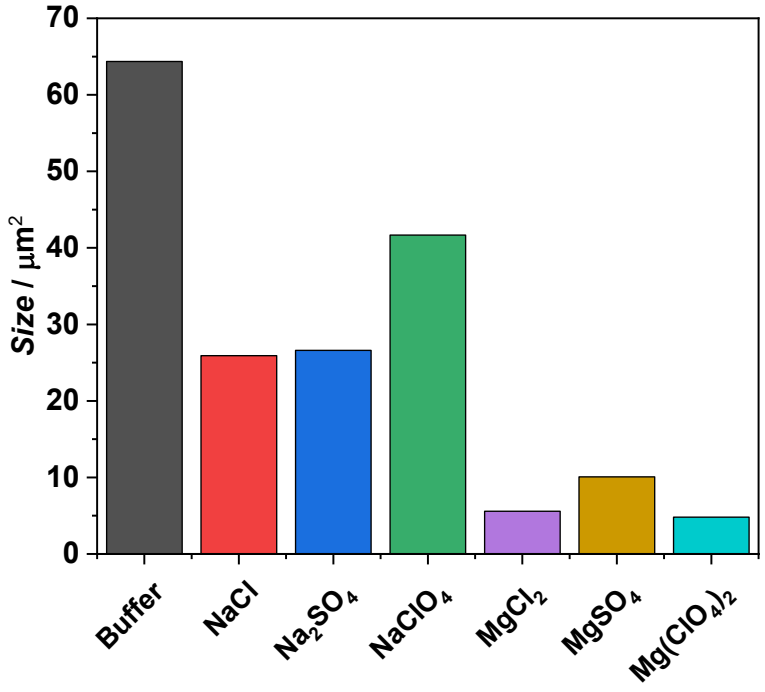

Figure S4 GUVs average size obtained from the lognormal distribution. All the experiments were performed in 10 mM HEPES buffer, pH 7.4, at the temperature of 35 °C.

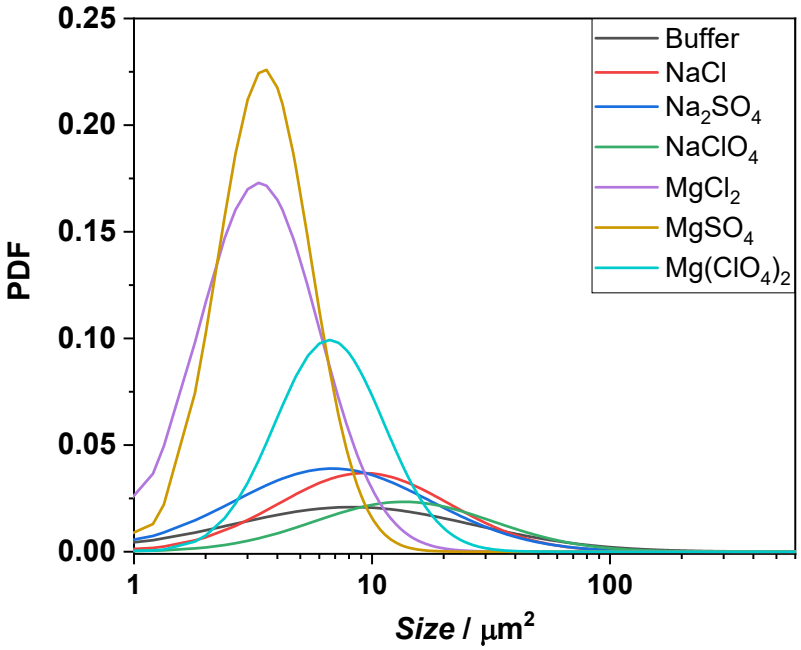

Figure S5 Normalized size distribution of GUVs in the presence of each Martian salt as indicated in the inset. All the experiments were performed in 10 mM HEPES buffer, pH 7.4, at the temperature of 35 °C.

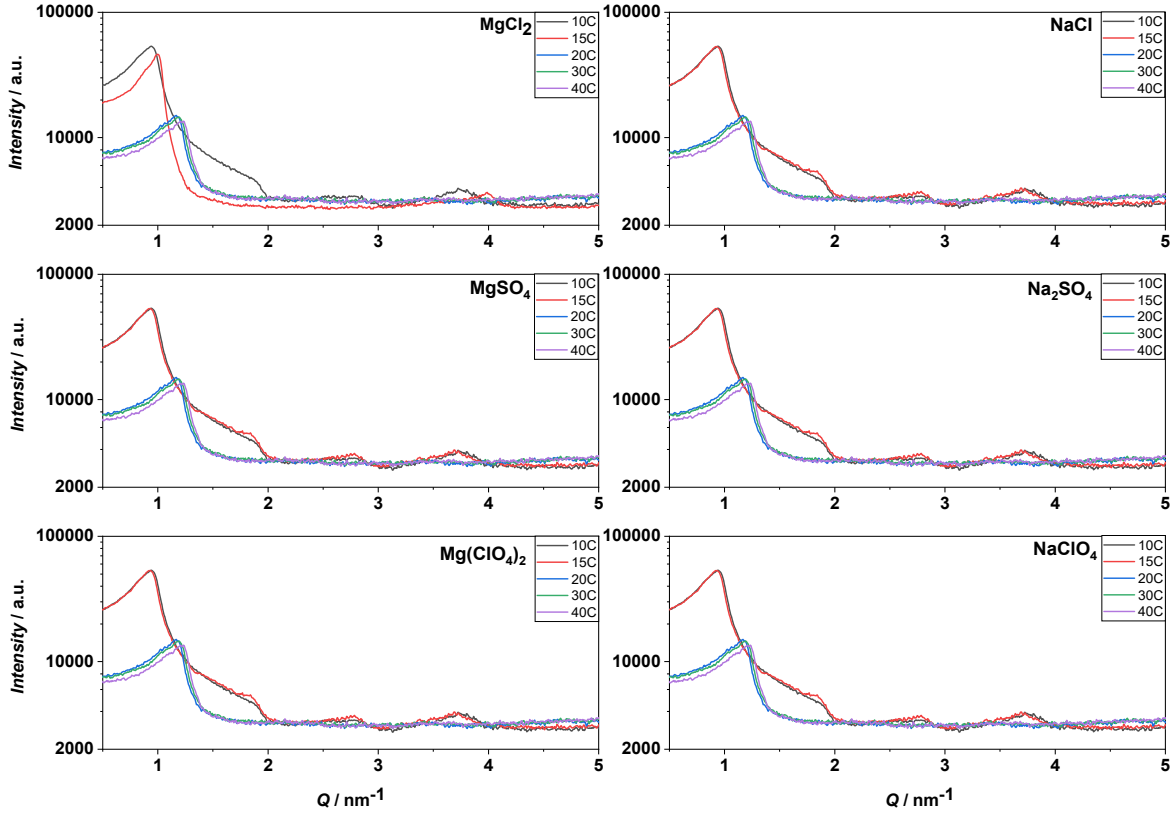

Figure S6 Temperature dependent SAXS curves of POPE/POPG 8/2 multilamellar vesicles in the presence of 0.5 M of different Martian relevant salts, as indicated in the insets. All the experiments were performed in 10 mM HEPES buffer, pH 7.4.

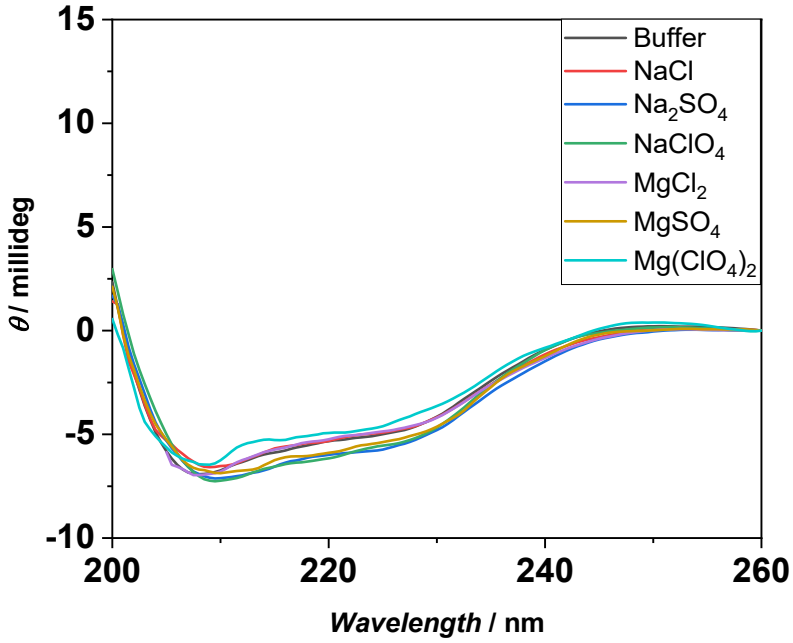

Figure S7 Far-UV CD spectra of the PLA2 protein in the absence and in the presence of 0.5 M of different Martian relevant salts at $T = 35$ °C. All the experiments were performed in 10 mM HEPES buffer, pH 7.4.

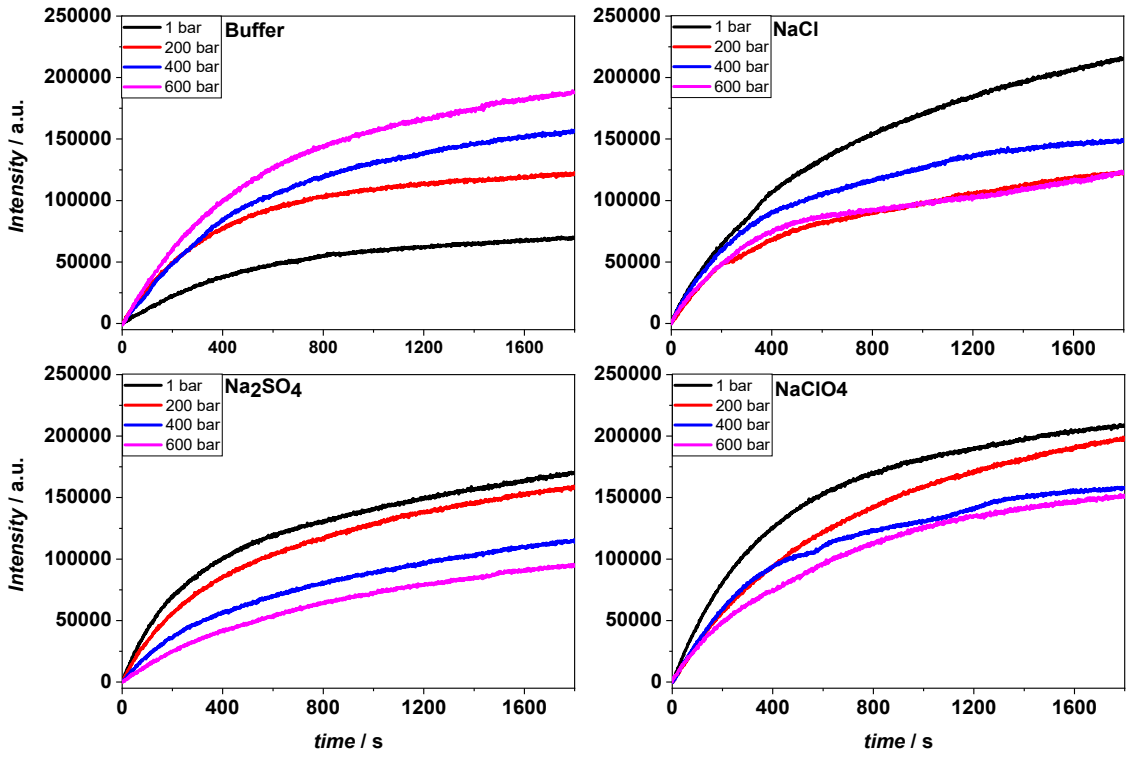

Figure S8 Pressure dependent kinetic plots of PLA2-induced hydrolysis of DBPC embedded in POPE/POPG vesicles under neat buffer conditions and in the presence of 0.5 M of the sodium salts indicated. All the experiments were performed in 10 mM HEPES buffer, pH 7.4, at $T = 35$ °C.

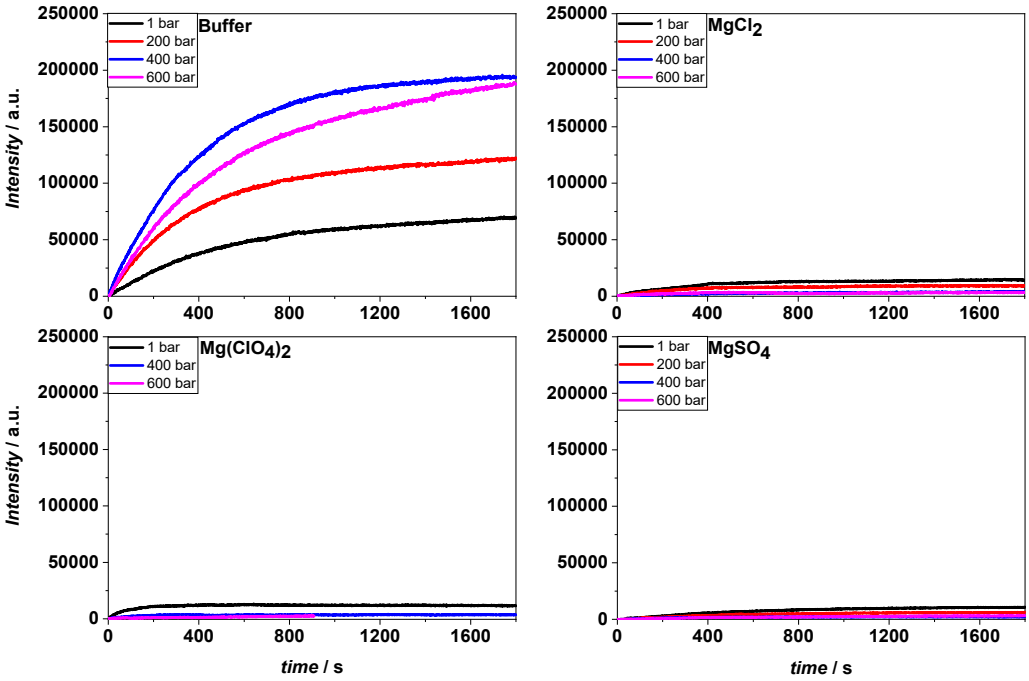

Figure S9 Pressure dependent kinetic plots of PLA2-induced hydrolysis of DBPC embedded in POPE/POPG vesicles under neat buffer conditions and in the presence of 0.5 M of the magnesium salts indicated. All the experiments were performed in 10 mM HEPES buffer, pH 7.4, at $T = 35$ °C.