Spontaneous Vesicle Formation of Monododecenyl Phosphonic Acid in Water

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Abstract: We report the synthesis of amphiphilic dodecenyl phosphonic acid PC12 from vinylphosphonic acid, a reactive phosphonic acid intermediate. The trans-P=C=C moiety enabled PC12 to disperse well in water. Surface tension and dynamic light scattering measurements revealed that PC12 exhibited high surface activity and reduced the surface tension of water from 72.0 to 23.6 mN/m, thereby resulting in the spontaneous formation of aggregates even in a dilute aqueous solution (critical aggregation concentration (CAC) = 4.8 × 10⁻⁴ M). In contrast to modern lipids with double-tailed structures, the PC12 of simple single-tailed structure spontaneously formed bilayered vesicles, without an external energy supply. Compared with the strength of hydrogen bonds formed by the long, saturated alkyl chain of dodecyl phosphonic acid (DPA), the strength of PC12 intermolecular hydrogen bonds was weaker. The melting point of PC12 was approximately 20°C lower than that of DPA. These results indicate that the trans-P=C=C moiety was considerably important for spontaneous vesicle formation in water. Preliminary modeling of the morphological transitions of the closed bilayer structures in the vesicles was then conducted, by varying the pH and adding an α-helical peptide scaffold.

Key words: phosphonic acid, surfactant, vesicle, origin-of-life

1 Introduction

The self-assembly of amphiphiles is a fundamental phenomenon in nature1-3. A significant example of this process is the self-assembly of the most abundant modern phosphonolipids. These phosphonolipids contain a phosphate in the polar head group and a glycerol backbone with a double-tailed structure, which collectively form the matrix of a biological membrane3. Origin-of-life studies have demonstrated that closed bilayer membrane structures, formed spontaneously from simple amphiphiles with a single-tailed structure, are regarded as the first protocells on the primitive Earth4,5,10.

The possible role of single-tailed phosphorus amphiphiles such as alkyl phosphates11-13 and phosphonic acids14,15 in protocell membranes has been debated. In contrast to simple organic amphiphiles such as fatty acids, they exhibit unusual phase behaviour, i.e., the formation of liquid crystalline phases over a wide pH range11-13. Furthermore, alkyl phosphonic acids were the only phosphorus-containing molecules10 found in the carbonaceous Murchison meteorite, which were successfully synthesized under plausible prebiotic conditions17. This suggests that the phosphorous-containing molecules potentially contributed to the formation of protocell membranes14-20. Although parts of ancient oceans might have a lower pH due to the activity of volcanoes and the hydrothermal vents21,22, typical alkyl phosphonic acids in non-dissociated form are generally less soluble in water owing to the formation of robust hydrogen-bonded networks23,24. Schulz et al.25 reported the binary phase diagram for the dodecyl phosphonic acid (DPA)/water system, which indicates that DPA starts to form lamellar liquid crystals at relatively high temperatures. Therefore, the process by which amphiphiles could form closed bilayer membrane structures in the ancient ocean without an external energy supply remains unclear.

This paper describes the spontaneous vesicle formation of closed binary membrane structures from monoalkenyl phosphonic acid, P(O)(OH)₂CH = CH(CH₂)₂CH₂(PC12). PC12 was catalytically synthesized from vinylphosphonic acid, a reactive phosphonic acid intermediate of a potent prebiotic phosphorus carrier17. We found that introduction...
of a trans-P–C = C moiety to the amphiphile significantly increased hydrophilicity, which enabled PC₁₂ to disperse well in water. Moreover, significant information about the self-assembling behavior of highly hydrophilic alkenyl phosphonic acid in water was obtained. The PC₁₂ vesicle formed spontaneously without any apparent energy input and remained stable for at least two months. Additionally, we performed preliminary modeling of the morphological transitions of the closed bilayer structures in the vesicles. Because the vesicles could experience gradual increasing of pH together with incorporating other biological ingredients during the evolution of complex cellular life, we also conducted the morphological transition experiments by varying the pH and adding an α-helical peptide scaffold.

2 Experimental Procedures

2.1 Materials

Diethyl vinylphosphonate (>98.0%, Tokyo Chemical Industry Co., Ltd.), Grubbs Catalyst™ 2nd Generation (Sigma Aldrich), 1-dodecene (>99.5%, Tokyo Chemical Industry Co., Ltd.), dichloromethane (super dehydrated, FUJIFILM Wako Pure Chemical Co., Ltd.), hexane (>96%, FUJIFILM Wako Pure Chemical Co., Ltd.), ethyl acetate (>99.5%, FUJIFILM Wako Pure Chemical Co., Ltd.), ethanol (>99.5%, FUJIFILM Wako Pure Chemical Co., Ltd.), potassium iodide (>99.5%, FUJIFILM Wako Pure Chemical Co., Ltd.), chlorotrimethylsilane (>98.0%, Tokyo Chemical Industry Co., Ltd.), acetoneitrile (>99.5%, FUJIFILM Wako Pure Chemical Co., Ltd.) and silica gel (Wakogel C-200, FUJIFILM Wako Pure Chemical Co., Ltd.) were used as received. AW21mer was synthesized using a previously reported method.²⁸⁻³⁰

2.2 Synthesis and characterization

¹H, ¹³C and ³¹P NMR spectra were recorded on an Avance 400 NMR spectrometer (Bruker Biospin Co., Inc.). Either CDCl₃ or D₂O was used as the deuterated solvent. Tetramethylsilane served as an internal reference for calibration of the ¹H and ¹³C NMR spectra obtained in CDCl₃. ¹H NMR spectra in D₂O were calibrated with the signal from the solvent residue. Phosphoric acid served as an external standard for ³¹P NMR. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) was conducted on an autoflex speed TOF/TOF MS (Bruker Daltonics Inc.) using 2,5-dihydroxybenzoic acid (DHB) as the matrix. Attenuated total reflection (ATR) Fourier transform infrared spectroscopy (FT-IR) was conducted on a Nicolet 6700 spectrometer (Thermo Scientific Inc.). Each sample was placed on the diamond crystal plate for measurement, and 32 scans were conducted from 400-4000 cm⁻¹. Spectral data were collected and processed with the OMNIC operating system (Version 7.3 Thermo Nicolet).

2.2.1 P(O)(OCH₂CH₃)₃CH = CH(CH₃)₂CH₃ synthesis

In a Schlenk flask equipped with a magnetic stir bar, 1-dodecene (2.5 g, 15.0 mmol) and diethyl vinylphosphonate (1.9 g, 11.3 mmol) were dissolved in 15 mL dichloromethane. Grubbs Catalyst™ 2nd generation (0.64 g, 0.80 mmol) was then added, and the mixture was refluxed for 3 h. The solvent was evaporated under reduced pressure, and the crude product was purified by silica gel chromatography (Rf=0.28) by eluting with 2:1 hexane:EtOAc followed by 1:1 hexane:EtOAc. P(O)(OCH₂CH₃)₃CH = CH(CH₃)₂CH₃ was obtained as a pale brown liquid (3.2 g, 10.5 mmol, 93%).

2.2.2 P(O)(OH)CH = CH(CH₃)₂CH₃(PC₁₂) synthesis

P(O)(OCH₂CH₃)₃CH = CH(CH₃)₂CH₃ (3.20 g, 10.5 mmol) and KI (10.5 g, 63.3 mmol) were charged in a two-necked round bottom flask with a magnetic stirring bar and dissolved in MeCN (40 mL). Me₃SiCl (6.88 g, 63.3 mmol) was added, and the solution was allowed to stir at room temperature for 10 h. The solvent was then removed under reduced pressure. Water (40 mL) was added, and the mixture stirred for 1 h. The water was partially evaporated, and the product was extracted into EtOAc to obtain a white solid (2.57 g, 1.56 mmol, 98%).

2.3 Surface activities and self-assembling properties

The surface tension measurements were performed by the Wilhelmy plate method at 25°C with a DY-500 surface tension meter (Kyowa Kaimen Kagaku Co.). Calibration was carried out prior to the measurement with ultra-pure water. The Pt plate was cleaned by flaming, and the glassware was rinsed multiple times with ultra-pure water. Aqueous solutions of PC₁₂ at various concentrations were maintained at 25°C overnight to allow equilibration. The light scattering intensities of the solutions were measured with a DLS-7000 instrument (Otsuka Electronics Co.) equipped with a 75-mW Ar laser (488 nm) light source at 25°C.

2.4 Observation of vesicles

An aqueous solution containing PC₁₂ and 10% methanol was quickly frozen with liquid nitrogen. The frozen sample was fractured in a JFD-V freeze fracture system (JEOL). The fractured surface was then replicated by platinum evaporation, followed by carbon coating at normal incidence to strengthen the replica. The surface was washed, and the replica was placed on a copper grid. It was then examined and photographed with a JEM-1010 (JEOL) transmission electron microscope. A BX41 polarized optical microscope (OLYMPUS) equipped with cross-polarizing filters, and a DP12 charge-coupled device camera (OLYMPUS) was also used to observe the PC₁₂ vesicles.

2.5 Titration experiments

Titrations requiring pH measurement were carried out...
with a F-74 pH meter (HORIBA Corp.), which was calibrated regularly using pH 4.0, 7.0, and 9.0 standard buffer solutions. Because PC\textsubscript{12} formed various self-assembling structures in water, a 2:1 (v/v) ethanol:water mixed solvent system was used to observe a clear end point. All the measurements were performed at room temperature. A fixed volume of PC\textsubscript{12} solution (0.01 M) in ethanol-water was prepared. To this solution, aqueous NaOH (0.1 M) was added in discrete amounts with continuous stirring. The pH was recorded continuously until equilibrium was reached before the next addition of titrant. The apparent pK\textsubscript{a} of PC\textsubscript{12} was estimated as the pH of the solution at half the neutralization volume, e.g., half the volume required to reach the neutralization end point.

2.6 Morphological transitions of vesicles

2.6.1 pH-induced morphological transitions

NaOH (1.0 or 2.0 equiv.) was added to 6.7 × 10^{-2} M aqueous solutions of PC\textsubscript{12}, and the samples were left at room temperature overnight. The obtained plate-like crystals and micelles were observed with a BX41 polarized optical microscope (OLYMPUS) and a DLS-7000 instrument (Otsuka Electronics Co.) equipped with a 75 mW Ar laser (488 nm) light source at 25°C.

2.6.2 α-helical peptide-induced morphological transitions

PC\textsubscript{12} was dissolved in PBS (pH 7.4), to which AW21mer was added. The concentrations of AW21mer and PC\textsubscript{12} in the solution were 1.0 × 10^{-3} M and 1.0 × 10^{-2} M, respectively. The solution was vortexed for 30 min, then incubated for 4 h at 25°C at 1000 rpm. The sample was allowed to sit for three days at room temperature. The concentration of dissolved AW21mer in the sample was measured by UV-visible spectrometry at an absorbance wavelength of 280 nm on a V-560 UV-vis spectrometer and a F-74 pH meter (Otsuka Electronics Co.) was used to observe a clear end point. All the measurements were performed at room temperature. A fixed volume of PC\textsubscript{12} solution (0.01 M) in ethanol-water was prepared. To this solution, aqueous NaOH (0.1 M) was added in discrete amounts with continuous stirring. The pH was recorded continuously until equilibrium was reached before the next addition of titrant. The apparent pK\textsubscript{a} of PC\textsubscript{12} was estimated as the pH of the solution at half the neutralization volume, e.g., half the volume required to reach the neutralization end point.

2.6.2 α-helical peptide-induced morphological transitions

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3 Results

3.1 Synthesis

The structure of PC\textsubscript{12} is shown in Fig. 1(a), in which a C=C bond is directly attached to a P atom to form a characteristic hydrophilic trans-P-C=C moiety. PC\textsubscript{12} was synthesized through a cross-metathesis reaction of P(O)(OCH\textsubscript{2}CH\textsubscript{3})\textsubscript{2}CH = CH\textsubscript{2} with CH\textsubscript{3} = CH(CH\textsubscript{2})\textsubscript{3}CH\textsubscript{3} in the presence of a Ru-carbene catalyst, (H\textsubscript{2}IMes)PCy\textsubscript{3}Cl\textsubscript{2}Ru = CH\textsubscript{3}PCy\textsubscript{3}Cl\textsubscript{2}H\textsubscript{2}Mes = N,N-bis (mesityl)-4,5-dihydroimidazol-2-ylene)\textsuperscript{29-31}. Refluxing the mixture in CH\textsubscript{2}Cl\textsubscript{2} for 3 h afforded P(O)(OCH\textsubscript{2}CH\textsubscript{3})\textsubscript{2}CH = CH(CH\textsubscript{2})\textsubscript{3}CH\textsubscript{3} in 93% isolated yield (Figs. S1-3). The subsequent hydration of esters was conducted in the presence of Me\textsubscript{3}SiCl and KI to give P(O)(OH)\textsubscript{2}CH = CH(CH\textsubscript{2})\textsubscript{3}CH\textsubscript{3}(PC\textsubscript{12}) as a white solid in 98% isolated yield (Figs. S4-7). The 1H NMR spectrum of PC\textsubscript{12} in CDCl\textsubscript{3} contained signals at δ 6.77 and 5.71, which were assigned to the vinylene hydrogens. The trans configuration of the C=C bond was confirmed by the large 3J-1H coupling constant (J = 17.6 Hz).

Although its structure and polarity were similar to those of typical fatty acids, the non-ionic form of DPA was less soluble in water as its water solubility of DPA was estimated to be 2.8 × 10^{-4} M based on the 1H NMR analysis. The excellent water dispersity of PC\textsubscript{12} was confirmed by temperature-dependent conductivity measurements, which showed that PC\textsubscript{12} did not precipitate even at 0°C. In contrast, the solubility of DPA, which does not contain a P-C=C moiety, decreased significantly below 41.5°C (Fig. S8).
3.2 Surface activities and self-assembling properties

Next, we confirmed the surface activity of the highly hydrophilic, non-ionic form of PC₁₂ in water, as well as its molecular assembly behavior. The measurements of the water surface tension revealed that it gradually reduced by PC₁₂ owing to the adsorption at the air-water interface, which eventually made the surface tension constant (Fig. 2). The critical aggregation concentration (CAC) and surface tension at this concentration (γ₁₂) were estimated from the crossover point of the two fitted lines (CAC = 4.8 × 10⁻⁴ M and γ₁₂ = 23.6 mN/m). When the air–water interface was completely occupied by PC₁₂, the excess molecules began aggregating. The relative scattering intensity suddenly increased at the CAC (Fig. 2), indicating that PC₁₂ started aggregating above this concentration. The pH values of the aqueous solutions of PC₁₂ at various concentraions exhibited no significant change (pH = 3.8 for 2.0 × 10⁻³ M, 2.7 for 2.0 × 10⁻⁴ M, 2.6 for 1.0 × 10⁻⁵ M, and 2.1 for 2.0 × 10⁻⁶ M), indicating PC₁₂ exists in the non-ionic form in water, regardless of its concentration. Concentration-dependent ¹H and ³¹P NMR spectra of PC₁₂ in D₂O are shown in Fig. 3(a and b). It can be seen that the peak corresponding to the CH₃ proton at the end of the hydrophobic group started shifting downfield (Fig. S9) above the CAC. Furthermore, when PC₁₂ concentration was above 3.3 × 10⁻³ M, the peak significantly broadened. A similar tendency was observed in the ³¹P NMR spectra, in which the peak corresponding to the phosphorus of the hydrophilic group significantly broadened above 3.3 × 10⁻³ M. Signal broadening is usually observed when phosphonolipids form bilayer structures, because the linewidths of the NMR spectra are sensitive to crystalline–liquid crystalline transitions of the hydrocarbon chains, as well as to the magnetic field strength and viscosity. Thus, we concluded that PC₁₂ formed micelles just above CAC (4.8 × 10⁻⁴ M) and also translate into different types of aggregates above 3.3 × 10⁻³ M.

The direct observation of a colloidal solution of PC₁₂ with freeze-fracture transmittance microscopy (FF-TEM) revealed that PC₁₂ self-assembled into large spherical particles, as seen in Fig. 3(c). The observation of the phase-contrast microscopy at a higher concentration (1.7 × 10⁻⁴ M) revealed the formation of spherical particles of varying size, which are shown in Fig. 3(d). We confirmed that the aggregates were vesicles with a lamellar bilayered structure through cross-polarised optical microscopy. Maltese crosses were observed, as shown in Fig. 3(e). The observation of large vesicles at higher concentrations suggests that the diameter of vesicles depends on the concentration. Notably, the vesicles were stable in water for two months at room temperature and did not precipitate, thereby demonstrating their thermodynamic stability. At a high concentration of 2.7 M (40 wt%), the sample exhibited an optically anisotropic texture (Fig. S10). This suggested the formation of lamellar liquid crystals. The subsequent evaporation of water results in thin plate-like crystals, which further confirmed that PC₁₂ tended to form bilayer structures (Fig. S11).

3.3 Morphological transitions of vesicles

We also studied the influence of the degree of PC₁₂ dissociation on self-assembly. An acid-base titration of PC₁₂ with NaOH was performed in a 2:1 (v/v) ethanol:water mixed solvent system (Fig. S12). The acid dissociation constants of PC₁₂ were estimated to be 3.9 (pK₁) and 9.3 (pK₂). Then, we conducted a titration in water. As shown in Fig. 4(a), the solution exhibited complex phase behav-
The solubility of 

PC₁₂

decreased significantly when 1.0 equiv. of NaOH was added, and thin plate-like crystals formed (Fig. 4(b)). Moreover, pH shifted from 2.1 to 6.6, indicating the presence of PC₁₂ of mono sodium salt. The addition of 2.0 equiv. of NaOH led to the formation of a colorless, transparent solution, whose pH increased to 10.6. The DLS measurements of the final diluted aqueous solution (9.2 × 10⁻³ M) indicated the formation of small aggregates with the hydrodynamic diameters of 31.2 ± 6.0 nm (Fig. 4(c)). The results indicated the PC₁₂ vesicles transformed into plate-like crystals and micelles depending on the pH. The same behavior was also observed for dodecyl phosphate of mono and di-potassium salts. The dodecyl phosphate of mono-potassium salt precipitated in water because of dimerization with that of non-ionic form as acid soap type complex. The dodecyl phosphate of di-potassium salt provided micelles owing to the electrostatic head group repulsions.

In biological systems, the morphological transitions of lipid vesicles often arise owing to chemical heterogeneity caused by processes such as the incorporation of proteins, carbohydrates, and peptides. These establish communication with the external environment, facilitate responsiveness to transport molecules, and enable certain metabolic functions. Peptides are of particular interest because amino acids can be readily synthesized by potentially prebiotic routes, suggesting that peptides were likely to have been present in the protocellular system. Notably, the PC₁₂ vesicles formed spontaneously without any apparent energy input. This allowed us to model the evolution of complex cellular life.

To understand the interactions between the bilayer structure of PC₁₂ with the peptides, we focused on amphiphilic α-helical scaffold peptides, including NH₂-VLES-FKASFLSALGKYKLN-NH₂ (AW21mer), that mimic helix 10 of the human apoA-I protein. Owing to the hydrophobic and hydrophilic and hydrophobic face, the peptides were found to interact with 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) vesicles with a lamellar...
bilayered structure to form nanodisks in phosphate buffered salt (PBS). Based on our previous work, a solution of PC_{12} in PBS (pH 7.4), was prepared at a concentration higher than its CAC ([PC_{12}] = 1.0 \times 10^{-3} \text{ M}). Based on the apparent pK values, PC_{12} existed as a monosodium salt with low solubility. Notably, the addition of AW21mer (1.0 \times 10^{-3} \text{ M}) solubilized PC_{12} to give a clear solution (Fig. 5 (a)). After centrifugation to remove the partially insoluble solids, we performed DLS measurements of the solution. The obtained histogram in Fig. 5 (b) shows that the size distribution contained two peaks with the average diameters of 76.2 \pm 10.5 and 229.6 \pm 58.9 nm. To separate the non-aggregated components, the solution was passed through a size-exclusion chromatography (SEC) column (Fig. S13). The SEC fraction corresponding to the major peak was collected, and an aliquot was taken for negative stain transmission electron microscopy (NS-TEM) analysis. In contrast to PC_{12}, small aggregates of various shapes and sizes were observed in the fraction (Fig. 5 (b), inset), which may have resulted from a major alteration of the membrane curvature caused by interactions between the AW21mers. It was still unclear why PC_{12} spontaneously formed various self-assembled structures in water, even though it had a single-tailed structure similar to the typical surfactants. We believe the trans-P-C = C moiety is the key factor that increased hydrophilicity. The double bond may also affect the critical packing parameter. The slightly vented trans-P-C = C moiety introduces disorder into the membrane owing to looser packing and weaker van der Waals interactions. The analysis of PC_{12} by infrared spectroscopy (IR) revealed a P = O peak (v_{P=O}) at 1259 cm\(^{-1}\), which shifted relative to that of DPA (1211 cm\(^{-1}\)), as shown in Fig. S14. This indicated the weakening of intermolecular hydrogen bonds between the P(O)(OH)\(_2\) moieties in the solid state\(^{30}\), which in turn lowered the melting point of PC_{12} (73-75°C) than that of DPA (90-95°C)\(^{40}\). It is reported that phosphatidylcholines with a cis double bond located approximately in the middle of the sn-2 chain exhibits ca. 50°C lower phase transition temperature, compared with the fully saturated one\(^{41}\). Moreover, the molecular dynamics simulations suggested the double bond position significantly influenced the lipid bilayer properties\(^{42}\).

These moderately strong hydrogen bonding interactions could induce the formation of a pseudo-double-tailed PC_{12} dimer structure, the cylindrical shape of which promoted the formation of flat bilayers\(^{30}\). However, in dilute aqueous solutions with concentrations slightly above the CAC, dimerization may have been inhibited by water acting as a competitive molecule. The single-tailed structure of PC_{12} can result in a micellar phase. PC_{12} at higher concentrations begins to form dimers. Once a certain concentration is reached, spontaneous vesicle formation would occur. The observation of the phase transition induced by the addition of NaOH suggested that hydrogen-bonding interactions had a role in the vesicle formation. Mixing PC_{12} with an α-helical scaffold peptide (AW21mer)/ in PBS (pH 7.4) changed the membrane curvature owing to the interaction between the PC_{12} lamellar bilayer and the hydrophobic face of AW21mers.

4 Conclusion

In conclusion, we synthesized amphiphilic dodecenyl phosphonic acid PC_{12} from vinylphosphonic acid, a reactive phosphonic acid intermediate. Owing to the trans-P-C = C moiety, PC_{12} showed remarkable water dispersity and did not precipitate. DPA, which is believed to be a potent primitive amphiphile, was less soluble in water. PC_{12} exhibited high surface activity. It reduced the surface tension of water from 72.0 to 23.6 mN/m and formed aggregates, even in a dilute aqueous solution (CAC = 4.8 \times 10^{-3} \text{ M}). In contrast to modern lipids with double-tailed structures, PC_{12} has a simple single-tailed structure, and it spontaneously forms bilayer vesicles without an external energy supply. Compared with the strength of hydrogen bonds formed by the long-saturated alkyl chain of DPA, the strength of PC_{12} intermolecular hydrogen bonds was weaker. Thus, the melting point of PC_{12} was approximately 20°C lower than that of DPA. These results suggested that the trans-P-C = C moiety was of significant importance for spontaneous vesicle formation in water.

We also demonstrated that the vesicles of PC_{12} underwent morphological transitions with changes in pH and the addition of an α-helical peptide scaffold. The importance of modeling studies is significant, because the plasma membrane is composed of roughly equal proportions of proteins and lipids. The model studies could be stipulated that vesicles formed around acidic volcanoes and/or hydrothermal vents could experience the gradual increase in pH along with incorporating peptides as fragments of proteins during the evolution of complex cellular life.

We explored the properties of a self-assembling system
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containing a mixture of PC_{12} and an α-helical peptide scaffold.

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Supporting Information

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References

1) Israelechvili, J.N.; Mitchell, D.J.; Ninham, B.W. Theory of self-assembly of hydrocarbon amphiphiles into micelles and bilayers. J. Chem. Soc. Faraday Trans. 2 72, 1525-1568 (1976).

2) Janiak, M.J.; Small, D.M.; Shipley, G.G. Temperature and compositional dependence of the structure of hydrated dimyristoyl lecithin. J. Biol. Chem. 254, 6068-6078 (1979).

3) Antonietti, M.; Forster, S. Vesicles and liposomes: a self-assembly principle beyond lipids. Adv. Mater. 15, 1323-1333 (2003).

4) Akbarzadeh, A.; Rezaei-Sadabady, R.; Davaran, S.; Joo, S.W.; Zarghami, N.; Hanifehpour, Y.; Samiei, M.; Kouhi, M.; Nejati-Koshki, K. Liposome: classification, preparation, and applications. Nanoscale Res. Lett. 8, 102-110 (2013).

5) Luisi, P.L. The Emergence of Life From Chemical Origins to Synthetic Biology. Cambridge University Press (2006).

6) Sakuma, Y.; Imai, M. From vesicles to protocells: the roles of amphiphilic molecules. Life 5, 651-675 (2015).

7) Chen, I.A.; Walde, P. From self-assembled vesicles to protocells. Cold Spring Harbor Perspect. Biol. 2, a002170 (2010).

8) Mansy, S.S.; Szostak, J.W. Thermostability of model protocell membranes. Proc. Natl. Acad. Sci. USA 105, 13351-13555 (2008).

9) Monnard, P.-A.; Deamer, D.W. Membrane self-assembly processes: steps toward the first cellular life. Anat. Rec. 268, 196-207 (2002).

10) Hargreaves, W.R.; Deamer, D.W. Liposomes from ionic, single-chain amphiphiles. Biochemistry 17, 3759-3768 (1978).

11) Streiff, S.; Ribeiro, N.; Wu, Z.; Gumieni-Kontecka, E.; Elhabiri, M.; Albrecht-Gary, A.M.; Ourisson, G.; Nakatani, Y. "Primitive" membrane from polypropylen phosphates and polypropenyl alcohols. Chem. Biol. 14, 313-319 (2007).

12) Pozzi, G.; Birault, V.; Werner, B.; Dannenmuller, O.; Nakatani, Y.; Ourisson, G.; Terakawa, S. Sigle-chain polypropylen phosphates from "primitive" membranes. Angew. Chem. Int. Ed. 35, 177-180 (1996).

13) Ourisson, G.; Nakatani, Y. The terpenoid theory of the origin of cellular life: the evolution of terpenoids to cholesterol. Chem. Biol. 1, 11-23 (1994).

14) Walde, P.; Wessicken, M.; Radler, U.; Berclaz, N.; Conde-Frieboes, K.; Luisi, P.L. Preparation and characterization of vesicles from mono-n-alkyl phosphates and phosphonates. J. Phys. Chem. B 101, 7390-7397 (1997).

15) Schulz, E.P.; Pinheiro, A.; Rodriguez, J.L.; Minardi, R.M.; Frechero, M.; Schulz, P.C. Intermediate structure for higher level arrangements: catching disk-like micelles in decane phosphonic acid aqueous solution. J. Phys. Chem. B 117, 6231-6240 (2013).

16) Cooper, G.W.; Onwo, W.M.; Cronin, J.R. Alkyl phosphonic acids and sulfonic acids in the Murchison meteorite. Geochim. Cosmochim. Acta 56, 4109-4115 (1992).

17) de Graaf, R.M.; Visscher, J.; Schwartz, A.W. Reactive phosphonic acids as prebiotic carriers of phosphorus. J. Mol. Evol. 44, 237-241 (1997).

18) de Graaf, R.M.; Visscher, J.; Schwartz, A.W. A plausible prebiotic synthesis of phosphonic acids. Nature 378, 474-477 (1995).

19) Schwartz, A.W. Phosphorus in prebiotic chemistry. Phil. Trans. R. Soc. B 361, 1743-1749 (2006).

20) Schwartz, A.W. Prebiotic phosphorus chemistry reconsidered. Orig. Life Evol. Biospheres 27, 505-512 (1997).

21) Corliss, J.B.; Baross, J.A.; Hoffman, S.E. An hypothesis concerning the relationship between submarine hot springs and the origin of life on earth. Oceanol. Acta No. SP, 59-69 (1981).

22) Ariga, K.; Yuki, H.; Kikuchi, J.; Dannenmuller, O.; Albrecht-Gary, A.M.; Nakatani, Y.; Ourisson, G. Monolayer studies of single-chain polypropenyl phosphates. Langmuir 21, 4578-4583 (2005).

23) Thomas, L.C.; Chittenden, R.A.; Hartley, H.E.R. Hydrogen bonding in alkyl phosphonic and alkyl phosphonothionic acids. Nature 192, 1283-1284 (1961).

24) Blanchard, J.W.; Groy, T.L.; Yarger, J.L.; Holland, G.P. Investigating hydrogen-bonded phosphonic acids with proton ultrafast MAS NMR and DFT calculations. J. Phys. Chem. C 116, 18824-18830 (2012).

25) Schulz, P.C.; Abrameto, M.; Puig, J.E.; Soltero-Martinez, F.A.; Gonzalez-Alvarez, A. Phase behavior of the...
systems n-decanephosphonic acid/water and n-dodecane phosphonic acid/water. Langmuir 12, 3082-3088 (1996).

26) Ikeda, Y.; Taira, T.; Sakai, K.; Sakai, H.; Shigeri, Y.; Imura, T.; Tsukui, Y.; Sakai, K.; Sakai, H.; Abe M., Kitamoto, D. Surfactant-like properties of an amphiphilic α-helical peptide leading to lipid nanodisc formation. Langmuir 30, 4752-4759 (2014).

27) Imura, T.; Tsukui, Y.; Taira, T.; Aburai, K.; Sakai, K.; Sakai, H.; Taira, T.; Kitamoto, D. Minimum amino acid residues of an α-helical peptide leading to lipid nanodisc formation. J. Oleo Sci. 63, 1203-1208 (2014).

29) Chatterjee, A.K.; Choi, T.-L. Grubbs, R.H. Synthesis of vinyl-and alkylphosphonates by olefin cross-metathesis. Synlett 1034-1037 (2001).

30) Han, L.-B.; Tanaka, M. Palladium-catalyzed hydrophosphorylation of alkenes via oxidative addition of HP(O)(OR)2 J. Am. Chem. Soc. 118, 1571-1572 (1996).

31) Xu, Q.; Han, L.-B. Metal-catalyzed additions of H-P(O) bonds to carbon-carbon unsaturated bonds. J. Organomet. Chem. 696, 130-140 (2011).

32) Cullis, P.R.; Hope, M.J. Effects of fusogenic agent on membrane structure of erythrocyte ghosts and the mechanism of membrane fusion. Nature 271, 672-674 (1978).

33) Sakai, T.; Miyaki, M.; Tajima, H.; Shimizu, M. Precipitate deposition around CMC and vesicle-to-micelle transition of monopotassium monododecyl phosphate in water. J. Phys. Chem. B 116, 11225-11233 (2012).

34) Huang, C.; Yuan, H.; Zhang, S. Coupled vesicle morphogenesis and domain organization. Appl. Phys. Lett. 98, 043702-043704 (2011).

35) Hirst, L.S.; Ossowski, A.; Fraser, M.; Geng, J.; Selinger, J.V.; Selinger, R.L.B. Morphology transition in lipid vesicles due to in-plane order and topological defects. Proc. Natl. Acad. Sci. USA 110, 3242-3247 (2013).

36) Patel, B.H.; Percivalle, C.; Ritson, D.J.; Duffy, C.D.; Sutherland, J.D. Common origins of RNA, protein and lipid precursors in a cyanosulfidic protometabolism. Nat. Chem. 7, 301-307 (2015).

37) Buchoux, S.; Lai-Kee-Him, J.; Garnier, M.; Tsan, P.; Besson, F.; Brisson, A.; Dufourc, E.J. Surfactin-triggered small vesicle formation of negatively charged membranes: a novel membrane-lysis mechanism. Biophys. J. 95, 3840-3849 (2008).

38) Kamat, N.P.; Tobé, S.; Hill, I.T.; Szostak, J.W. Electrostatic localization of RNA to protocell membranes by cationic hydrophobic peptides. Angew. Chem. Int. Ed. 54, 11735-11739 (2015).

39) Thomas, L.C.; Chittenden, R.A. Characteristic infrared absorption frequencies of organophosphorus compounds-I The phosphoryl (P=O) group. Spectrochimica Acta 20, 467-487 (1964).

40) Ghassamipour, S.; Sardarian, A.R. Friedländer synthesis of poly-substituted quinolines in the presence of dodecyl phosphonic acid (DPA) as a highly efficient, recyclable and novel catalyst in aqueous media and solvent-free conditions. Tetrahedron Lett. 50, 514-519 (2009).

41) Coolbear, K.P.; Berde, C.B.; Keough, M.M.W. Gel to liquid-crystalline phase transitions of aqueous dispersions of polyunsaturated mixed-acid phosphatidylcholines. Biochemistry 22, 1466-1473 (1983).

42) Martinez-Seara, H.; Rög, T.; Pasenkiewicz-Gierula, M.; Vattulainen, I.; Karttunen, M.; Reigada, R. Effect of double bond position on lipid bilayer properties: insight through atomistic simulations. J. Phys. Chem. B 111, 11162-11168 (2007).