DOI: 10.1002/anie.200905465

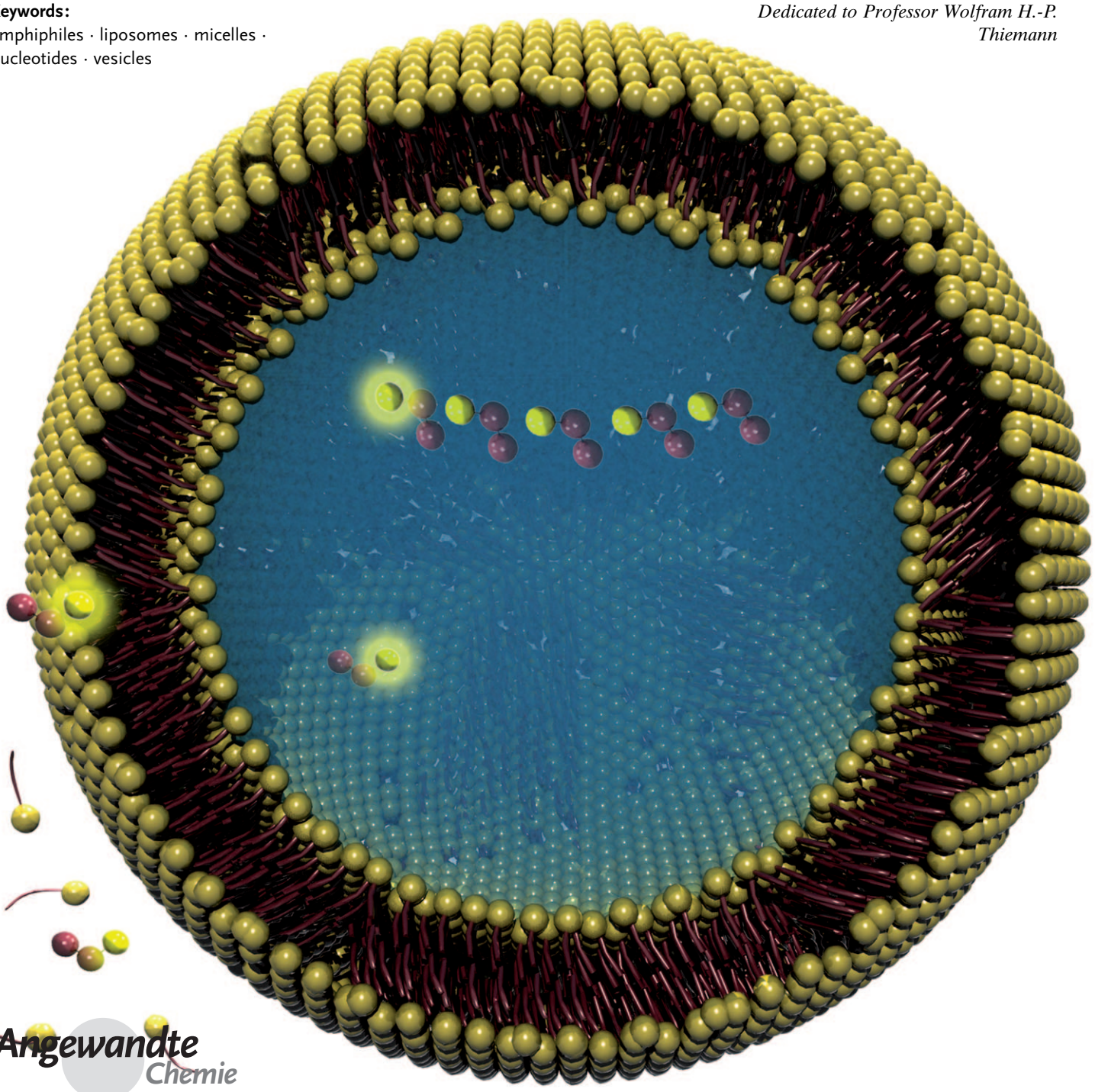
The Origin of Life

On the Origin of Primitive Cells: From Nutrient Intake to Elongation of Encapsulated Nucleotides

Uwe J. Meierhenrich, Jean-Jacques Filippi, Cornelia Meinert, Pierre Vierling, and Jason P. Dworkin*

Keywords:
amphiphiles · liposomes · micelles · nucleotides · vesicles

Dedicated to Professor Wolfram H.-P. Thiemann



Recent major discoveries in membrane biophysics hold the key to a modern understanding of the origin of life on Earth. Membrane bilayer vesicles have been shown to provide a multifaceted microenvironment in which protometabolic reactions could have developed. Cell-membrane-like aggregates of amphiphilic molecules capable of retaining encapsulated oligonucleotides have been successfully created in the laboratory. Sophisticated laboratory studies on the origin of life now show that elongation of the DNA primer takes place inside fatty acid vesicles when activated nucleotide nutrients are added to the external medium. These studies demonstrate that cell-like vesicles can be sufficiently permeable to allow for the intake of charged molecules such as activated nucleotides, which can then take part in copying templates in the protocell interior. In this Review we summarize recent experiments in this area and describe a possible scenario for the origin of primitive cells, with an emphasis on the elongation of encapsulated nucleotides.

From the Contents

1. Introduction

Cells are the basic units of all current life forms. In typical modern prokaryotic and eukaryotic cells, a compartment-defining phospholipid bilayer—which also contains glycolipids and steroids, including cholesterol—separates the fluid outside from the inside of the cell. The cell's interior contains a well-defined variety of biological compartments and molecules, and it is here that the RNA machinery expresses the genetic code into functional proteins. The phospholipid bilayer consists of two hydrophilic surfaces and a hydrophobic interior, which prevents polar molecules such as amino acids, nucleic acids, phosphorylated carbohydrates, proteins, and ions from entering the cell through the wall without an enzymatic control mechanism. Thus, modern cells, which are composed of hundreds of different membrane lipids,[1] require sophisticated protein channels and energy-dependent pumps to mediate the exchange of molecules with their environment. However, can modern biochemistry decipher the mechanism for the origin of cells and their membranes at the time that primitive life started its biological evolution on Earth? Acquiring this knowledge constitutes a long-standing research goal, both from a fundamental perspective and in view of the potential applications of artificial cells.

Biochemical evidence suggests that cells are important for the appearance of life, allowing for the encapsulation, concentration, and protection of (in)organic molecules from the external prebiotic "soup" of diluted (in)organic nutrients, and also allowing for chain growth and template copying reactions in their interior. An understanding of the prebiotic evolution of bilayer membrane vesicles is hence at the center of general debates on the origin of life on Earth. However, there is a nagging problem: phospholipid membranes are highly effective barriers to polar and charged molecules, necessitating complex channels and pumps to permit the exchange of molecules with the external environment. Contemporaneous phospholipid membranes are nonpermeable to a large variety of molecules essential for cell life, growth, and multiplication, and lack the dynamic properties required for both membrane growth and the intake of nutrients. Understanding of the spontaneous formation of primitive cell-like vesicles from amphiphilic molecules, nutrient intake through the lipid membrane bilayer, and elongation of encapsulated nucleotides inside model-cell systems has advanced dramatically in recent years. In this Review we consider these fascinating steps from the viewpoint of chemists and biochemists.

2. Self-Assembly of Amphiphiles into Cell-like Vesicles: A Primitive Cell in the Laboratory

Molecules that self-assemble from a disordered state to form vesicular cell-like structures have attracted scientific interest for decades. These surface-active molecules[2] require an amphiphilic character, which means that polar and nonpolar functional groups are present in the same molecule. Fatty acids and fatty alcohols serve as typical examples of

[*] Prof. Dr. U. J. Meierhenrich, Dr. J.-J. Filippi, C. Meinert, Dr. P. Vierling
LCMBA UMR 6001 CNRS, Institut de Chimie de Nice
Université de Nice-Sophia Antipolis
Faculté des Sciences, Parc Valrose, 06108 Nice (France)
Fax: (+33) 4-9207-6151
E-mail: Uwe.Meierhenrich@unice.fr
Homepage: http://www.unice.fr/lcmba/meierhenrich/
Dr. J. P. Dworkin
Astrochemistry Laboratory, Code 691.0
NASA Goddard Flight Space Center
Greenbelt MD 20771, Maryland (USA)

Supporting information for this article including a 3D video on primitive cell formation is available on the WWW under http://dx.doi.org/10.1002/anie.200905465.

molecules with self-assemply capabilities that can spatially orient neighboring molecules. Phospholipids in the cells of modern organisms as well as amphiphilic zwitterionic gemini surfactants[3,4] also show these characteristics.

As a consequence of the outstanding advances made in understanding the dynamic properties of fatty acid aggregates for the origin of life, we will focus on fatty acid vesicles, keeping in mind that these vesicles require both relatively high concentrations and particular physicochemical stimuli to form. Modern phospholipid amphiphiles require concentrations that are up to six orders of magnitude lower than those of fatty acids to self-assemble into vesicles.

Fatty acids and fatty alcohols are commonly found in experiments simulating the prebiotic "soup". These amphiphiles can be synthesized under prebiotic conditions, as long as the molecules are chemically relatively simple and do not need to be enantiomerically pure.[2] Two distinct pathways for the formation of amphiphiles have been described in topical theories on the origin of life: one related to geophysical sites, such as marine hydrothermal systems, and another to extraterrestrial sources, such as the protosolar nebula, which were fed by interplanetary and interstellar nebulae. The chemical analysis of each provides individual characteristic challenges.

2.1. Fischer–Tropsch Synthesis of Amphiphilic Molecules in the Aqueous Phase

The Fischer–Tropsch reaction has attracted the attention of geochemists as a potential starting point for the formation of organic molecules, including amphiphiles. The Fischer–Tropsch reaction is known to occur in different geological settings, such as volcanoes and igneous rocks. For a long time, it was assumed that the Fischer–Tropsch process could not occur in the aqueous phase because of inhibition by water, but recent laboratory experiments by Simoneit and co-workers have proven that the chemical formation, accumulation, and selection of amphiphiles is feasible by Fischer–Tropsch reactions even in the aqueous phase.[5,6] Fischer–Tropsch synthesis in the aqueous phase is important since mid-ocean-ridge hydrothermal systems are increasingly being discussed as a possible starting place for the origin of life on Earth. This arises from the discovery of primitive life forms around hydrothermal vent systems at the bottom of the ocean, where magma (liquid rock) spills through the Earth's crust and reacts with sea water.

Contemporaneous marine hydrothermal systems, however, are dominated by organic compounds derived from all-pervasive biological processes; thus experimental simulations provide the best opportunity for confirmation of the potential for organic synthesis in such systems. Consequently, Fischer–Tropsch reactions have been performed in the laboratory under controlled temperatures and pressures that mimic hydrothermal conditions. Starting with aqueous solutions of either formic or oxalic acid (as substitutes for CO, CO_2, and

Uwe J. Meierhenrich studied chemistry at the Philipps University of Marburg. After completing his PhD at the University of Bremen, he identified amino acids in artificial comets at the Max-Planck Institute for Solar System Research in Katlenburg-Lindau and at C.B.M. in Orléans in preparation for the cometary Rosetta mission. In 2005, he was promoted to full Professor at the University of Nice-Sophia Antipolis. His book, "Amino Acids and the Asymmetry of Life", was published in 2008.

Cornelia Meinert received her diploma in chemistry in 2004 at the University of Leipzig, where she focused on organic and environmental chemistry. She is currently completing her PhD studies on preparative capillary GC with Werner Brack at the Helmholtz Centre, and became a postdoctoral fellow in the group of Uwe Meierhenrich at the University of Nice-Sophia Antipolis. Her research interests focus on the origin of biomolecular asymmetry, especially enantiomer separation by using GCxGC techniques.

Jean-Jacques Filippi studied natural product chemistry at the University of Corsica. He moved to the University of Nice-Sophia Antipolis in 2000, where he obtained his PhD in 2005. After postdoctoral research at the University of Hohenheim in the team of H. Strasdeit on prebiotic chemistry, in 2006 he became assistant professor at LCMBA. His current scientific interests focus on flavors and fragrances and prebiotic chemistry.

Pierre Vierling studied chemistry at the University of Strasbourg where he gained his PhD. He joined the CNRS in 1979 at the University of Nice-Sophia Antipolis (UNS). He was promoted to Research Director in 1996 and is currently head of the LCMBA. His current scientific interests focus on gene (DNA) delivery systems with a particular interest in highly fluorinated systems for "artificial viruses" and for the specific delivery of DNA to targeted cells.

Jason P. Dworkin began research into the origins of life with Joan Oró at the University of Houston, where he studied amino acids and co-enzymes. He completed his PhD in biochemistry under Stanley Miller at UCSD, where he investigated pre-RNA nucleobases. He then carried out postdoctoral research at NASA Ames and founded the Astrobiology Analytical research group at NASA Goddard Space Flight Center. He is currently Chief of the Astrochemistry Branch at NASA Goddard.

H_2 in hydrothermal fluids to overcome the practical difficulties of adding these volatile gas components to the high-pressure reaction vessel) as the carbon and hydrogen sources, the formation of lipid compounds with carbon chains between C_2 and C_{35} in length, including *n*-alkanols and *n*-alkanoic acids, was observed inside reaction vessels after cooling, extraction, and GC-MS analysis. The identification of the reaction products was confirmed using ^{13}C-labeled reactants. Both formic and oxalic acid carbon sources yielded the same lipid classes with essentially the same ranges of compounds. The optimum temperature window for the formation of alkanoic acids was 300 °C; higher temperatures reduce the yield because of competing cracking processes.[6] Table 1 presents the relative concentrations and range of carbon chain lengths of alkanoic acids obtained by Fischer–Tropsch synthesis at various temperatures in the aqueous phase.

Table 1: Alkanoic acid carbon number ranges of products of aqueous Fischer–Tropsch reactions at different temperatures.[6]

	100 °C	150 °C	200 °C	250 °C	300 °C	350 °C	400 °C
range	7–9	7–22	7–13	7–16	7–18	7–13	7–18
C_{max}[a]	7	7	9	7	7	7	8
rel. concentration[b]	–	7	7	8	20	6	4
CPI[c]	–	0.98	1.14	1.15	1.05	1.07	0.95

[a] C_{max} = carbon number of most abundant alkenoic acid. [b] In μg 100 μg^{-1} extract. [c] Carbon preference index, $CPI = \Sigma(C_9 + C_{11} + C_{13} + C_{15} + C_{17} + C_{19} + C_{21} + C_{23})/\Sigma(C_8 + C_{10} + C_{12} + C_{14} + C_{16} + C_{18} + C_{20} + C_{22})$.

Carbon preference index (CPI) values vary from 0.95 to 1.15, and show no predominance of a particular carbon number. CPI values close to one indicate that the chain growth of the homologue series is by single carbon units. The Fischer–Tropsch reaction in the aqueous phase thus proceeds by the transformation of oxalic acid to C_1 species such as CO, followed by insertion of the CO group at the terminal end of a growing carboxylic acid to form homologous series of alkanoic acids after reduction.[6] This mechanism differs from the classically known industrial Fischer–Tropsch process, in which the growth of the hydrocarbon chain relies on the reaction of vapor-phase mixtures of CO or CO_2 with H_2 through surface-catalyzed stepwise polymerization of methylene.[5,6] Besides amphiphilic molecules, straight-chain alcohols, alkyl formates, aldehydes, ketones, alkanes, and alkenes were identified as products of the Fischer–Tropsch reaction in the aqueous phase. Methyl alkanes were generated at $T >$ 250 °C, with a maximum concentration at 350 °C. As a result of the hydrothermal Fischer–Tropsch mechanism, the identified molecules have a linear structure, and only minor quantities of branched and cyclic hydrocarbons form.[5] The formation of branched alkanoic acids was not reported.

The synthesis of amphiphiles under hydrothermal conditions has been demonstrated by Hazen and Deamer,[7] who subjected pyruvic acid (which can also be synthesized under hydrothermal conditions) to hydrothermal processing. Chemical analysis of the products and specific surface-activity tests showed that chain lengths between 2 and 18 carbon atoms were present in the synthesized products, which dispersed slowly into large numbers of microscopic spherical structures with apparent internal compartments, as shown by epifluorescence microscopy. The synthesized products were able to self-assembly into vesicular structures.

Interestingly, the synthesis of amphiphilic lipid compounds readily occurs under prebiotic hydrothermal conditions.[8] It has been assumed that the accumulation of amphiphilic lipids can lead to the generation of not only micelles but also membrane-like vesicles in aqueous environments and thus provide precursor substrates for protocells,[5,6] as will be outlined in the following sections.

The hypothesis of the origin of living cells triggered by Fischer–Tropsch reactions in the aqueous phase has raised particular interest because several lines of evidence indicate that early forms of life were hyperthermophiles that developed in geothermal regions such as hydrothermal vents. It should be emphasized that this opinion is not universally shared.[9] Deciphering the molecular architecture of the first cell-like vesicles from today's molecular anamnesis of hyperthermophiles (sometimes called the top-down approach) remains a difficult task since all contemporaneous hyperthermophiles have highly specialized lipid components evolved by enzymatic pathways, and it seems likely that these are the result of more recent adaptation than a molecular fossil of early life.[10]

2.2. Interplanetary and Interstellar Synthesis of Amphiphilic Molecules

The infall of extraterrestrial material to the early Earth is also considered a source of bilayer-forming compounds. Besides amino acids[11–13] and precursors of biological cofactors,[14] amphiphilic molecules of eight or more carbon atoms have been identified in simulated precometary ices.[15] Precometary ices can be produced in high-vacuum chambers in the laboratory by mimicking the interstellar environment in terms of temperature, pressure, as well as vacuum ultraviolet or proton irradiation and observing the presence of gas-phase molecules condensing on a substrate over several days. Milligrams of simulated precometary ices are hence precious sources for chemical analysis, which provide information on the primitive material from which the solar system formed. The arrival of extraterrestrial compounds—as the assumption goes—contributed to the functional organic inventory of early Earth and triggered the appearance of life. Molecules detected in simulated precometary ices could potentially play a significant role in prebiotic chemistry, including the evolution of the first cell-like vesicles.

After extraction of simulated precometary ices with methanol/chloroform, the mixture of extracted molecules was spotted on a microscope slide, dried, and alkaline sodium phosphate buffer added to obtain pH 8.5. The chosen

conditions were identical to the conditions[16] under which organic compounds extracted from carbonaceous meteorites produced a variety of self-assembled structures.[15] The molecules produced assembled into water-insoluble droplets and foams (≤ 50 μm in diameter) with different morphologies (Figure 1). Dworkin et al. concluded from various physico-

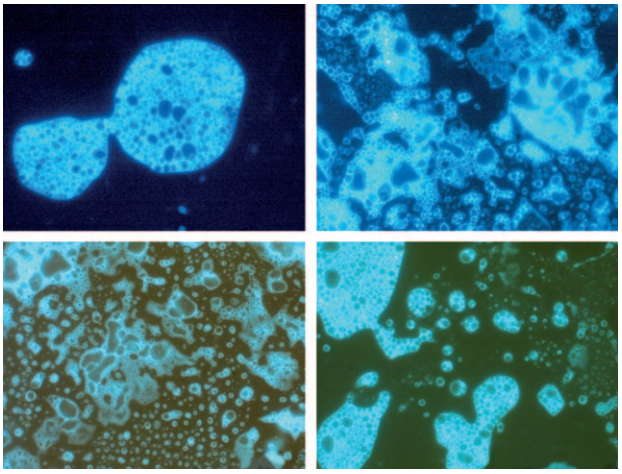


Figure 1. Residue droplets of a simulated precometary ice at pH 8.5 viewed by fluorescence microscopy and with 100-fold enlargement. The precometary ice was simulated by 0.8 MeV proton bombardment of amorphous ices of H_2O, CH_3OH, NH_3, and CO (100:50:1:1) at 15 K in a high-vacuum chamber.[17] The gas composition was chosen as a simple mixture that reflects the composition and concentrations of the major components of interstellar ice. The four images were recorded with different filters and show different areas of the extract.

chemical measurements on the mixture that their lipophilic chains contained at least eight carbon atoms.[15] Further experiments with an encapsulated dye confirmed that the amphiphilic components of the droplets assembled into membrane vesicles to provide well-defined interior spaces.[15]

2.3. Identification of Amphiphiles in Carbonaceous Meteorites

Functional organic molecules have been extracted from the carbonaceous Murchison meteorite. Murchison belongs to the CM2-type meteorites, several percent of the mass of which is known to be organic carbon. The meteorite has a complex history and certainly does not have the identical chemical composition as the simulated precometary ices presented in Section 2.2. However, in the case of the Murchison meteorite, enantioenriched amino acids,[13,18–20] chiral and achiral diamino acids,[21] nucleic bases,[22–23] amphiphilic molecules, and bolaamphiphile dicarboxylic acids[24] have been identified. Chloroform/methanol extracts of the meteorite sample showed that vesicles appear when a phosphate buffer is added to the organic extract. To determine whether the amphiphilic components can assemble into membranous vesicles with interior spaces, Dworkin et al. added a hydrophilic pyranine dye by a standard dehydration/rehydration cycle[25] (see Section 4.1) to an extract of the Murchison meteorite.[15] As shown in Figure 2, besides oil

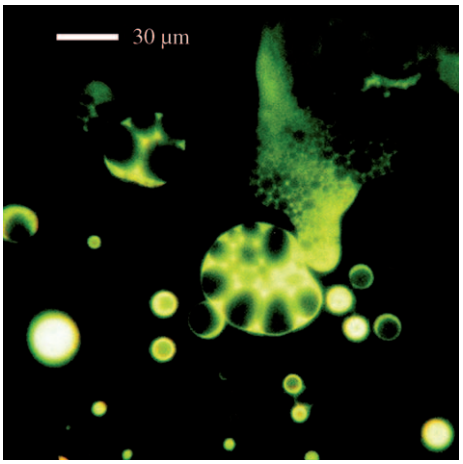


Figure 2. Compounds from meteorites seen in a new light: pyranine dye encapsulated in vesicles made from an extract of the Murchison meteorite. Vesicles show interior spaces with sizes in the micrometer range; oil droplets and inverse emulsions are also visible.[15] Copyright (2001) National Academy of Sciences, USA.

droplets and other morphologies, micrometer-sized vesicles were formed that encapsulated the fluorescent pyranine dye in their interior spaces. The exact composition of the membrane-forming amphiphiles was not established in this study because of the limited quantities of the Murchison meteorite extracts.[10]

Studies by Pizzarello and co-workers with solid-phase microextraction (SPME) sample preparation showed that low-molecular-weight monocarboxylic acids are the most abundant water-soluble organic compounds in the Murchison meteorite as well as in many other carbonaceous meteorites.[26] More than 50 monocarboxylic acids were identified in 11.3 g taken from the inside of the meteorite, quantities that are 10 to 100 times greater than those of amino acids. Compound-specific isotopic analyses performed with isotope ratio gas chromatography including a combustion system (GC-c-IRMS) offer new opportunities to better define the origins and formation pathways of organic compounds in meteorites. These studies showed δ(D) and $\delta(^{13}C)$ values that verify an interstellar origin of the amphiphilic molecules.[26] Besides linear-chain monocarboxylic acids with carbon chains up to C_{10}, a large range of randomly substituted branched-chain monocarboxylic acids was identified. This complex mixture of branched monocarboxylic acids was proposed to have originated by the exothermic and thermodynamically favored interstellar gas-phase radical reactions that take place between 10 and 100 K. More than 30 years ago, comparatively primitive analytical studies of the Murray and Murchison CM2 carbonaceous meteorites identified 18 monocarboxylic acids, which are identical to the core analytes detected by Pizzarello and co-workers.[27]

In 1989, extracts from the interior of a 90 g sample of Murchison meteorite showed evidence for surface activity involving both the formation of monomolecular films at air–water interfaces and self-assembly into membrane-containing vesicles with encapsulated polar solvents.[16] In this study, amphiphilic molecules extracted from the Murchison meteorite were chemically identified. These amphiphilic molecules

showed lipidlike behavior and self-assembled into vesicles. These findings suggest that extraterrestrial materials could exhibit a far greater range of chemical properties and behaviors than previously thought.[15] Amphiphilic molecules could have been delivered to planetary surfaces such as the early Earth, where they mixed with endogenous compounds synthesized on the planet.[10]

The relevance of fatty acid vesicles to origin of life scenarios lies in the fact that they are chemically simple versions of amphiphiles (in contrast to phospholipids used in contemporary biological cells). We conclude that fatty acids and other amphiphilic compounds present in carbonaceous meteorites can participate in self-assembly processes that lead to the formation of membranes, as can carboxylic acids synthesized by Fischer–Tropsch reactions under aqueous conditions.[28]

2.4. Designing the First Cell: Self-Assembly of Amphiphiles into Cell-like Vesicles

Amphiphilic molecules in which a single saturated hydrocarbon chain is linked to a polar head group will, when dispersed in an aqueous phase, self-assemble into different phases depending on the concentration, chain length, headgroup characteristics, and environmental factors, such as temperature, counterions, and pH value. Amphiphiles such as medium- and long-chain monocarboxylic acids, alcohols, amines, alkyl phosphates, and alkyl sulfates,[1] as well as organic–inorganic nanoparticle hybrid systems[29,30] typically form spherical micelles above the Krafft temperature[31] and above the critical micellar concentration (cmc). These amphiphiles can form bilayers and vesicles at a critical concentration for vesicle formation (cvc, sometimes abbreviated cbc for critical bilayer concentration)[32,33] in rapid dynamic equilibrium with single molecules and micelles. The cvc is usually much higher than the cmc. Free amphiphiles (that is, not bound in micelles or vesicles) are always present together with micelles and vesicles.[33]

Lipid vesicles, also called liposomes (strictly speaking, liposomes are vesicles made out of lipids),[34] or often simply vesicles,[35] are quasispherical shells composed of lipid bilayers that encapsulate an aqueous phase.[36–38] Unilamellar and multilamellar vesicles are generally formed upon dispersion of amphiphilies (or mixtures thereof) that self-assemble in water into lamellar phases. These quasispherical supramolecular structures are composed of thousands to millions of individual molecules,[1] with diameters ranging from 20 nm to 100 μm.[2] The structural similarity of unilamellar vesicles to the cell membrane has resulted in them being considered precursor structures or cell-mimicking compartments.[2] They are referred to as "protobionts", "probionts",[39] "protocells",[40] and "progenotes"[41,42] to ambitiously suggest "artificial cells".[43] It is assumed that these precursor structures are simpler than the first cells, perhaps much smaller than the smallest bacterium.[43]

A simplified ternary phase diagram for sodium octanoate, octanoic acid, and water is depicted in Figure 3.[44] Lamellar structures (and consequently vesicles) occur only in region D,

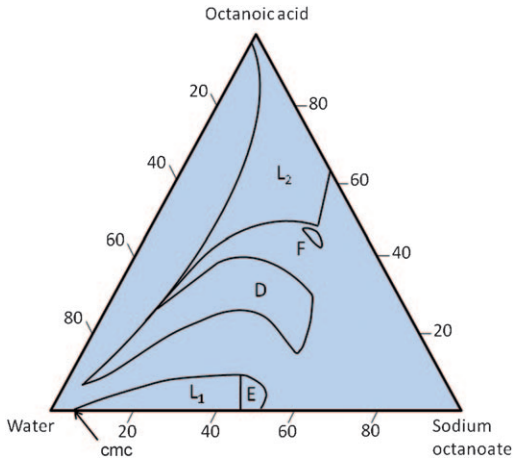


Figure 3. Ternary phase diagram for the sodium octanoate, octanoic acid, and water system at 20 °C expressed in wt %. The isotropic solution region L_1 represents an aqueous solution, and the isotropic solution region L_2 represents the solution of sodium octanoate and water in octanoic acid. E and F (liquid-crystalline two-dimensional hexagonal phase regions) are normal and inverse, respectively. The lamellar liquid-crystalline region D occurs in the center of the diagram. The phase diagram of the ternary system was shown to be very similar for longer chain length fatty acids as well as for the potassium carboxylate, carboxylic acid, and water system.[44]

that is, if both the sodium octanoate and octanoic acid forms are present. Amphiphilic single-chain carboxylic acids indeed form vesicles if about half of the amphiphilic molecules are present in the anionic form and half of the molecules are present in the protonated, non-ionic form,[33] hence typically if the pH value in the vesicles is close to the pK_a value of the carboxylic acid group.[28] The formation of intermolecular hydrogen bonds between protonated and ionized carboxylates has been proposed to explain the stability of carboxylic acid vesicles;[45,46] these bonds decrease the electrostatic repulsion between adjacent head groups. The stability of aggregates of amphiphilic molecules held together by hydrogen-bonding interactions has been confirmed by measurements of protonated and ionized carboxylate clusters in the gas phase.[47] Vesicle membranes are stable in the pH range where protonated and nonprotonated forms coexist. Micelles form at higher pH values, while oil droplets condense at lower pH values.[32,46,48] At room temperature, nonanoic acid forms, for example, stable vesicles at concentrations of 85 mM and pH 7.0, which corresponds to the pK_a value of the acid in the bilayers.[10] This concentration is, however, relatively high compared to the micromolar concentrations of various modern phospholipids required to form vesicles. Below pH 6, the carboxylate group of nonanoic acid is protonated, and the vesicles become unstable. The absence of protonated carboxylates above pH 8 results in the formation of micelles and the loss of vesicles.

The addition of other simple amphiphiles such as fatty alcohols[32] and fatty acid glycerol esters[49] allowed the further stabilization of fatty acid vesicles in a wider pH range, even in the presence of divalent cations. The addition of small amounts of nonanol to the nonanoic acid system described above results in the formation of hydrogen bonds between

hydroxy and carboxy groups. This allows the vesicles to now form at lower concentrations of about 20 mM at pH values ranging from 6 up to 11; thus, the vesicles are stabilized in the alkaline pH range.[32] Even if the vesicular membrane stabilizing system is more complex, and van der Waals interactions between the hydrocarbon chains, hydrophobic interactions, and solvent effects occur, this observation supports the above assumption that the stability of bilayer membranes increases as the pH-driven hydrogen bonding between adjacent head groups increases. Further stabilization of vesicles in the alkaline pH range was observed by Namani and Deamer[28] with a decylamine/decanoic acid system, in which the auxiliary decylamine acts as a hydrogen-bond donor. The pH range for vesicle formation can also be shifted to acidic pH values by the addition of surfactants such as sodium dodecylbenzene sulfonate (SDBS) to decanoic acid,[33] or by adding the auxiliary decylamine to the decylamine/decanoic acid system, which acts as a hydrogen-bond donor in the acidic pH range.[28]

The bilayer structure of pure saturated fatty acids has been observed to be unstable against divalent cations such as Mg^{2+}, Ca^{2+}, and Fe^{2+}. The addition of alkyl amines to fatty acids, such as decylamine to decanoic acid, was shown to produce bilayer structures that were resistant to the effects of divalent cations up to 0.1M.[28] This is an important finding since catalytic RNAs usually require significant concentrations of Mg^{2+} ions. Chen et al. described a catalytic RNA acting inside a vesicle formed from myristoleic acid ((*Z*)-9-tetradecenoic acid) and glycerol monoester. They found that this divalent-cation-tolerant vesicle is stable at Mg^{2+} concentrations that allow RNA catalysis.[50]

As the chain length of the lipophilic tail increases, the cmc and cvc decrease, and the stability of the vesicle consequently increases.[10] Saturated monoalkyl carboxylic acids with chain lengths of C_{13} and longer also form bilayers, but only if their hydrocarbon domains are maintained in a fluid state, that is, at a temperature above the crystal-to-liquid-crystal phase-transition temperature.[28]

We conclude that amphiphilic molecules can assemble into membranes and vesicles over a wide pH range through hydrophobic interactions as well as van der Waals and hydrogen-bond interactions between adjacent molecules.[46] The addition of alcohols, amines, and even polyaromatic hydrocarbons can stabilize vesicular structures. Further investigations into vesicles should concentrate on mixtures of amphiphiles and their response to different chemical and physical stimuli.

3. Divide et Impera: Growth and Division of Primitive Cellular Compartments

Once cell-like vesicles are formed by the self-assembly of amphiphilic molecules into spherical bilayers, they are observed to grow and divide under physical and chemical conditions that can be easily monitored under laboratory conditions. The controlled growth of primitive cell-like vesicles composed of fatty acids was observed by incorporating additional fatty acids by slowly adding amphiphiles or micelles to the external medium.[35,51–53] This phenomenon is not surprising and arises from the lyotropic phase behavior of the fatty acid in water. The growth process thus takes place as long as the final concentration of the fatty acid remains within a concentration range that is compatible with the existence of lamellar region D shown in Figure 3. Such a growth process of cell-membrane-like bilayers is driven by the rapid equilibrium between individual amphiphiles, micelles, and bilayers, which results in uptake of the amphiphiles and micelles by the bilayer structure and the concomitant dissolution of the micelles.

In principle, the simplest mechanistic models for the growth of carboxylic acid vesicles would be: 1) the direct fusion of micelles with vesicles in a single step, 2) the dissolution of micelles into carboxylic acids followed by incorporation into the preformed membrane, or 3) fusion of vesicles.[48] The first studies to decipher the mechanisms of growth and division of fatty acid vesicles were performed in Zurich by Luisi and co-workers.[52] Walde et al. reported an increase in the diameter of vesicles formed from oleic acid ((*Z*)-9-octadecenoic acid) and oleate after increasing the concentration of the amphiphilic molecules in the spherical boundary of the vesicles.[52] Increases in the vesicle size and number were observed, and since this process took place in the boundary of the parent vesicles, it was defined as an autopoietic self-reproduction.[52,53]

3.1. Vivat, Crescat, Floreat: Vesicle Growth

Cryotransmission electron microscopy (cryo-TEM) was applied in the first pioneering study that clearly demonstrated the growth of vesicles after the addition of fatty acid micelles.[35] Here, the water-soluble protein ferritin, which, because of its dense iron core, can be detected by cryo-TEM, was entrapped in the internal aqueous phase of preformed vesicles. The size distribution of filled (ferritin-containing) and empty vesicles could be distinguished, and the cryo-TEM data—obtained from frozen vesicle suspensions—gave evidence for the growth of vesicles upon the addition of fresh surface-active molecules, as well as evidence of the fission of larger vesicles, which led to a large number of small vesicles. Unfortunately, this cryogenic method could not be used to follow the growth of membrane vesicles in real time.[48]

Recently, Szostak and co-workers applied an innovative method based on membrane-localized fluorescence resonance energy transfer (FRET) dyes to follow the growth of fatty acid vesicles to distinguish between vesicle growth by direct micelle–vesicle fusion and vesicle growth by incorporation of free molecular fatty acids. A membrane-localized FRET donor–acceptor pair allowed the increase in the vesicle surface area to be measured during the controlled growth of vesicles by the careful addition of micelles. The FRET efficiency decreased as the surface density of the FRET dyes decreased on incorporation of additional fatty acid. In contrast to former experimental approaches, this method had the advantage of allowing for 1) the quantitative measurement of the growth of preformed vesicles even when new vesicles were formed simultaneously and 2) such measure-

ments to be made in real time during the process of controlled formation of the membrane.[48] Kinetic data revealed that none of the three mechanistic models of vesicle growth mentioned at the start of Section 3 is appropriate, and a new pathway involving previously unsuspected intermediate aggregates was proposed. The structure of these metastable intermediates could not be elucidated; candidate structures are bilayer patches, cuplike membrane structures, and long cylindrical micelles. The hydrodynamic radius of the heterogeneous intermediate aggregates could be determined by dynamic light scattering to be about 45 nm, much larger than that of spherical micelles.[48]

A time-resolved study on the micelle-to-vesicle transition of a different phospholipid/bile salt system had shown that intermediate metastable states occur, which were described as cylindrical wormlike micelles, which finally evolve via disks into vesicles.[54] Membrane patches and discs were reported to be short-lived intermediates in a micelle-to-vesicle transition in a model bile system,[55] and cuplike particles or open bilayers partially rolled into lipid tubules were identified during the formation of vesicles by the elastic bending energy approach.[56] The spontaneous formation and growth of vesicles in a micelle solution was studied by small-angle neutron-scattering experiments (SANS), thus opening up the possibility for experiments with a resolution of a few hundred milliseconds. These data revealed that cylindrical micelles form before their continuous transition into vesicles in the phospholipid/bile salt system.[57] In a similar manner, the sodium bis(2-ethylhexyl)sulfosuccinate (AOT) system showed that the number of micelles required to produce a vesicle is about 25–50.[58] Studies on the phase behavior of the reverse transition from vesicles to micelles by cryo-TEM also revealed that not only spherical micelles but long cylindrical micelles also form as intermediate nanostructures during the solubilization of phospholipid vesicles by surfactants.[59] The phospholipid and AOT systems mentioned here behave differently than the previously mentioned fatty acid systems.

The formation of vesicles was also observed to be mediated by minerals. It was shown that montmorillonite clay[43] as well as different minerals and surfaces such as quartz, pyrite, and gold nanostructures[60] accelerated the conversion of fatty acid micelles into bilayer membrane vesicles. Even silica particles with diameters of 6 nm, a diameter smaller than the smallest possible vesicle, promoted the formation of vesicles. Nucleation most likely involved the formation of small patches of membrane that can continue to grow at their edges independently of the silica spheres. This type of surface-assisted formation of vesicles was observed in real time, thus enabling the formation of vesicles streaming off a microsphere to be observed just after micelle addition.[60] The authors assumed that a layer of positively charged cations associated with or adjacent to the montmorillonite surface attracts negatively charged micelles or free fatty acid molecules, thereby increasing their concentration locally and thus facilitating their aggregation into a bilayer membrane.[51]

Chen et al. demonstrated that the osmotic pressure can coordinate the growth of a fatty acid vesicle as well as the potential growth of a self-replicating system inside the vesicle.[61] In-streaming monomers were trapped inside the vesicle by polymerization into RNA, thereby raising the osmotic pressure and causing the vesicle to grow. In this study, more efficient RNA replication provided faster cell growth.[61]

3.2. Dynamic Properties of Vesicles

In contrast to micelles, membrane vesicles are described as systems not at chemical equilibrium. They are thermodynamically unstable, and require energy to form.[34] In recent years it became more evident that non-equilibrium structures appear at all levels in biological systems, and, as Kondepudi and Prigogine stated, "*we cannot describe Nature around us without an appeal to nonequilibrium situations.*"[62] In this context it was shown that different size populations of vesicles can coexist for several days in the same solution without a tendency to fuse. The different vesicle sizes correspond to energy minima, but no tendency for a homogeneous size distribution was observed after mixing. However, the individual amphiphilic molecules were observed to be in local equilibrium with the vesicular structure. Cheng and Luisi concluded that two populations of different vesicle sizes can not only coexist, but also—because of higher uptake rates of amphiphilic monomers present in the surrounding solution by larger vesicles—compete with each other, for example, for the uptake of reagents.[34]

Vesicles composed of fatty acids, fatty alcohols, and fatty acid glycerol esters were shown to be thermostable and could maintain their molecular contents even when heated above 80 °C.[63] Bilayer vesicles are dynamic systems, and individual molecules can easily enter and leave the vesicular structure. Fatty acids in a bilayer membrane are in rapid exchange with the aqueous environment. Amphiphilic monomers can exchange from two different layers within one vesicle.[1] They were observed to flip from the outer shell into the inner shell and vice versa.[64] This behavior would be important for the intake of nutrients and the release of metabolites from cell-like vesicles through the bilayer membranes.

3.3. A New Generation of Cells: Controlled Vesicle Redivision

In the absence of the complex machinery that controls the division of modern cells,[65,66] the redivision of growing vesicles must rely on the intrinsic properties of the vesicle and the physicochemical forces of the environment.[46] In research and development, where vesicles are used as model membranes, and in pharmaceutical applications, where vesicles are applied as nanoscale containers for drug transport and delivery,[67] the most widely used method to prepare vesicles under controlled conditions in the laboratory is by extrusion of vesicle suspensions through small-pore filters. For "division", a vesicle enters a membrane pore under pressure, transforms into a cylindrical shape, and fragments into smaller vesicles with a diameter similar to the pore diameter, depending on the ratio of the vesicle size to the pore diameter.[37] Even though this method is widely applied, the

actual mechanism by which vesicles break up into smaller vesicles remains unclear.[37,38]

Szostak and co-workers distinguishes between two distinct mechanisms for vesicle division: 1) the parent vesicle can be broken into smaller membrane fragments, which subsequently reseal to form a new generation of smaller vesicles, or 2) by the pinching-off of smaller vesicles, thereby resulting in insignificant dilution of the vesicle contents.[51] A fluorescent dye (calcein) was, therefore, encapsulated into 90 nm sized myristoleate vesicles grown to a size of 140 nm through slow micelle addition, then extruded through 100 nm pores to a final mean size of 88 nm. It was found that 55 % of the dye had been lost from the vesicles during extrusion.[51] The results show that division of the myristoleate vesicles proceeds with only a slightly greater loss of internal contents than that required by the geometric constraints of deriving two daughter spheres from one larger parent.

In advanced studies Szostak and co-workers repeated cycles of growth and division by growing a population of extruded myristoleate vesicles by slow feeding with myristoleate micelles and then dividing by extrusion.[51] The amount of encapsulated calcein was followed after each growth period and each extrusion. As expected, essentially no dye was lost during any of the five growth phases, whereas 40 % of the dye was lost after each extrusion. These experiments constitute a proof-of-principle demonstration that vesicle growth and division can result from simple physicochemical forces, without any complex biochemical machinery.[51] Furthermore, environmental shear forces can cause vesicles to divide.[46]

It is interesting to note that when small amounts of fatty acids were added to pre-added vesicles, the final size distribution of the vesicles was close to the size of the pre-added vesicles, a phenomenon called "matrix effect".[68,69] These studies stimulated research on the effect of the distribution of mixed phospholipid/oleate vesicles on the size distribution of newly formed unilamellar vesicles. The regulation of the size distribution of newly formed vesicles was dependent on the amount of oleate added to preformed vesicles.[70]

In 2008 a scenario was presented in which the replication of a template inside a cell-like vesicle followed by the random segregation of the replicated genetic material leads to the formation of daughter protocells (see Section 5).[64]

4. Towards the Dynamics of Life: Nutrient Uptake through Bilayer Membranes

4.1. Encapsulation during Vesicle Formation by Dehydration/Rehydration

Successfully integrating functional chemical systems into the interior space of vesicles is a key challenge in biophysics.[43] Dehydration/rehydration is one of the most efficient encapsulation methods and allows nutrients and functional target molecules to be sequestered into the interior space of vesicles during vesicle formation. Such a process might well have triggered the appearance of cell-type vesicles on the early Earth.

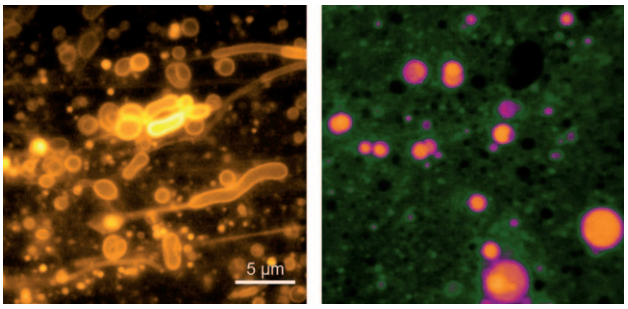


Figure 4. Left: Decanoic acid/decanol vesicles stained with fluorescent rhodamine; right: 600 mers of DNA encapsulated in vesicles of decanoic acid alone by the dehydration/rehydration method. The DNA was stained with 3,6-dimethylaminoacridine (acridine orange), a nucleic acid selective stain used to enhance the contrast in the microscopic image. Reprinted with permission from David Deamer, UC Santa Cruz.

Recent studies have shown that vesicles made from a decanoic acid/decanol mixture are capable of encapsulating and retaining a variety of organic macromolecules such as fluorescent dyes (Figure 4). The formation of vesicles in the presence of a dye resulted in the capture of the dye molecules within the vesicles. Subsequent size-exclusion chromatography allowed the separation of the vesicles from unencapsulated dye, thus releasing dye-enclosing vesicles for further investigations.[32] Not only dyes but also enzymes, such as catalase, and oligonucleotides can be encapsulated in fatty acid vesicles by using the dehydration/rehydration method (Figure 4).[25,32]

As described in Section 3.1, montmorillonite accelerated the conversion of fatty acid micelles into vesicles. The surface-mediated organization of the bilayer membrane allowed for the vesicular encapsulation of catalytically active surfaces such as montmorillonite. By previously loading the montmorillonite surface with adsorbed RNA, the RNA oligonucleotides were incorporated into the vesicles.[51] The observed encapsulation of mineral particles within vesicles thus introduced the catalytic potential of the RNA-labeled mineral surfaces into the vesicle.

Photoactive semiconducting particles, such as titanium dioxide particles in the 20 nm size range, were incorporated into vesicles by the dehydration/rehydration method. The particles retained their photoactivity and allowed incident light to drive photoelectrochemical reactions in a comparable manner to contemporaneous photosynthesis, and possibly relevant to the origin of life on Earth.[71]

4.2. The Static Solubility/Diffusion Theory

Phospholipid membranes of extant biological cells show limited permeability to ionic nutrients such as amino acids, nucleotides, and phosphate with measured permeability coefficients $P \approx 10^{-12}$ cm s^{-1}.[72] Deamer et al.[10] thus raised the question: "*how might an early form of cellular life gain access to nutrient solutes?*" We are confronted with the paradoxical situation that require vesicular membranes to be permeable enough to enable the intake of nutrients and to

also act as a barrier that prohibits the loss of the encapsulated primitive catalytic and genetic system. Without such a barrier, newly synthesized substances would diffuse into the surrounding bulk phase, and the potential for interactive systems and speciation would be lost.[73] Membranes in a fluid (liquid-crystalline) state rather than in a gel (crystal) state should be used to increase the membrane permeability for dissolved solutes. Another solution is to reduce the membrane thickness. These goals can be achieved by reducing the length of the lipophilic chains in the membrane-constituting amphiphiles,[10,74] by introducing *cis* double bonds or branching in the chains, and/or by adding amphiphiles with larger head groups.[64]

Various mechanisms have been proposed to describe the uptake of nutrients through bilayer membranes. The static solubility/diffusion theory interprets the bilayer membrane as a liquid hydrocarbon phase separating two aqueous phases. Permeating molecules will partition into the hydrophobic region, diffuse across, and leave by redissolving in the opposite aqueous phase. This process is driven by the concentration gradient and is also known as the passive diffusion mechanism. Permeability coefficients can hence be calculated if appropriate partition and diffusion coefficients as well as the membrane thickness are known. The solubility/diffusion theory is applicable for uncharged molecules, because of their relatively high solubility in the intermediate hydrocarbon phase. This theory also explains that uncharged amino acid methyl esters permeate lipid bilayers orders of magnitude faster than their zwitterionic parent compounds: amino acids are much less lipophilic than their methyl esters. Transmembrane pH gradients are used for active and quantitative loading into vesicles, and are also based on concentration gradients.[72]

4.3. The Dynamic Pore Mechanism

Discrepancies between predicted and measured permeabilities were observed for small ions penetrating thinner bilayer membranes. The alternative dynamic pore mechanism suggests that the permeation of ions through bilayer membranes occurs through pores or cavities that are hydrated transient defects produced by thermal fluctuations within the bilayer and cause disturbances in the lipid packing order.[75] Small ions can enter into these pores located in the headgroup region of the amphiphiles and pass through such hydrated defects, thereby evading the high-energy barrier associated with partitioning into the hydrophobic membrane interior.[74] If the membranes are sufficiently thin, the pores provide the dominant permeation pathways for ions. Ionic substrates such as the nucleoside triphosphate ATP were shown to permeate vesicular bilayers based on dimyristoylphosphatidylcholine (DMPC) at the gel-fluid main-phase transition temperature of 23.3 °C, at rates capable of delivering an encapsulated template-dependent RNA polymerase.[73] Permeation was observed to be greatest at the phase-transition temperature. At 37 °C, the optimal temperature for many enzyme-catalyzed reactions, the permeability decreased by two orders of magnitude.

The flip-flop mechanism could not be excluded for explaining the observed results, even if the authors envisaged the dynamic pore mechanism for ATP permeation. As an alternative to the dynamic pore mechanism, charged molecules can coordinate on the external shell of the vesicular membrane to the polar head groups of the amphiphilic molecules. These amphiphiles can flip from the outer/inner shell into the inner/outer shell where they are capable of releasing the charged molecules to the interior/exterior space of the vesicles (see Section 3.2.). This dynamic flip-flop phenomenon is most important at the main phase-transition temperature of the bilayer and in the fluid state (rather than the gel state). It is also highly influenced by the chemical properties (hydrophobicity, polarity of the head group) of the flipping amphiphile molecule. For example, the protonated fatty acids with $t_{1/2}$ flipping rates in the millisecond range are more dynamic than the more polar negatively charged carboxylates[76] and phospholipids ($t_{1/2} >$ days).[77] For further examples see the review article of Hamilton[76].

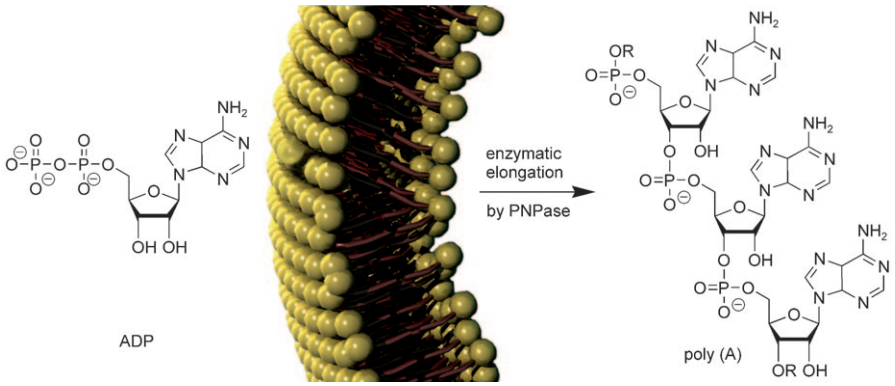
The functional enzyme catalase was encapsulated in decanoic acid/decanol vesicles, and its substrate, hydrogen peroxide, was added to the external aqueous environment. The bilayer membrane was shown to be permeable to hydrogen peroxide, with oxygen released inside the vesicle. The catalytic function of the catalase was maintained and the enzyme protected in the vesicular internal space against external influences, for example, catalase-degrading protease.[32] Similarly, polymerase enzymes encapsulated with their substrates in a cell-like vesicle led to polymeric products, which were protected from degradation by hydrolytic enzymes present in the external medium.[73] Walde et al. entrapped PNPase enzymes in oleic acid/oleate vesicles, followed by the external addition of ADP. The nutrient ADP, which carries three negative charges at pH 9, was observed to permeate across the vesicular bilayer into the interior space, where PNPase catalyzed the formation of poly(A), a stretch of ribonucleic acid, which was retained inside the membrane vesicle (Scheme 1).[45]

We have seen that under well-defined physicochemical conditions, amphiphilic molecules can form a population of bilayer membrane vesicles that "replicate" through processes of growth and division and have the ability to entrap macromolecules while remaining permeable to smaller polar solutes.[16,48] The dynamic pore and flip-flop mechanisms might have allowed early cells to have access to functional ionic nutrients from the external environment.

5. Non-Enzymatic Elongation of Encapsulated Nucleotides inside Cell-like Vesicles

In 2003, it was assumed that the encapsulation of mineral particles within membrane vesicles enables the use of the catalytic potential of the mineral surface for the elongation of encapsulated nucleotides.[51]

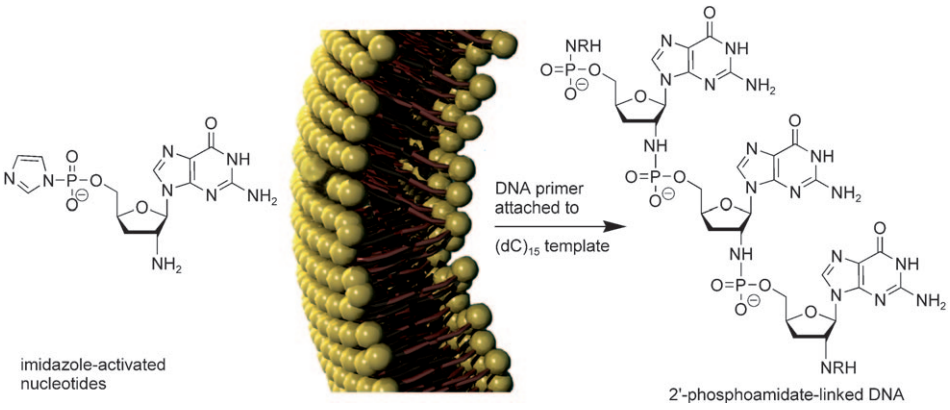
In 2008, elongation of an encapsulated genetic polymer was observed inside cell-like vesicles with neither a mineral surface nor enzymatic support. Synthetic single-strand DNA molecules with cytosine bases were trapped inside membrane



Scheme 1. ADP permeates across the vesicular bilayer into the interior space of oleic acid/oleate vesicles. Intra-protocellular enzymatic ADP elongation, catalyzed by polynucleotide phosphorylase (PNPase), results in poly(A), which stays in the extracellular medium.[45]

organisms or systems incorporated carbon-containing nutrients already available in the environment. The authors argue that early protocells made of fatty acid membranes could not have been autotrophs because internally generated metabolites would leak out.[64] Cellular life might first have sourced energy and nutrients from the environment, and more complex autotrophic lifestyles might have appeared at a later stage of evolution.[78] These experimental data again highlight that fatty acid membrane vesicles seem to be a suitable model for a protocell during early evolution leading to cellular life.[73]

Cellular evolution continued to progress. A typical protocell is assumed to encapsulate vesicles, and acted as primers and templates for their own elongation. Activated nucleotides containing the complementary guanosine bases were added to the surrounding medium of the vesicles. The mixture of molecules composing the vesicle membranes, including carboxylic acids, their corresponding alcohols, and monoglycerides, was optimized for maximal permeability to ribose, the sugar component of RNA, but minimal permeability to polymers such as DNA.[78] An elongation of the synthetic DNA primer was observed in the optimized cell-like vesicles as guanosine-containing imidazole-activated nucleosides were added one by one to the external medium. In contrast, no elongation was observed in parallel experiments with 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) vesicles.[64] The authors assumed that permeation of the imidazole-activated and negatively charged nucleotide across the membrane was driven by the interaction of its polar functional group with the amphiphile head group, whereas nonpolar regions of the nutrient interacted with the hydrophobic chains of the amphiphiles. The amphiphile–nutrient complex then flips from the outer to the inner membrane shell (see Section 4.3), carrying the nutrient to the internal space of the vesicle. This experiment shows that prebiotically plausible membranes composed of fatty acids provide surprisingly high permeabilities to charged molecules such as nucleotides, which can thus be incorporated from an external source of nutrients to take part in efficient template copying in the interior of the protocell (Scheme 2).



Scheme 2. Negatively charged imidazole-activated nucleotides cross the vesicular membrane and participate in non-enzymatic copying of an oligo-dC DNA template. Membrane vesicles were composed of decanoic acid, decanol, and decanoic acid glycerol monoester.[64]

Even though imidazole-activated nucleotides were certainly not provided by a prebiotic environment, the decoded non-enzymatic elongation of encapsulated nucleotides inside protocells may have far-reaching consequences: heterotrophic origin of life might have been feasible and early living not only an information-bearing template but also a polymerase or replicase composed of amino acids, so that sequence information in the template can be transcribed to a functional molecule.[10] Recently, oligopeptide synthesis from amino acid monomers inside vesicles made of fatty acids or phospholipids in a simulated hydrothermal environment was reported. It was found that encapsulation of the glycine monomers enhanced oligomerization.[79] For polymerase and replicase architecture, amino acid nutrients are required to cross the membrane barrier and enter the interior space of the cell-like vesicles. Controlled conditions that not only allow for the passage of charged nucleotides but also the uptake of zwitterionic amino acids while retaining polymerized nucleic acids inside vesicles will hopefully enhance our understanding of the crucial steps in the origin of life.

A first experimental approach for the synthesis of a minimal cell combined the reproduction of an oleic acid/oleate vesicle membrane with the simultaneous replicase-assisted replication of internalized RNA.[80] Discussions were ongoing regarding whether replicases, RNA synthesis, and membrane vesicles would grow and divide when fed with amphiphiles and precursors for membranes, and whether improved replicases[81] would evolve.[46] Szostak et al. pointed

out that a vesicle carrying an improved replicase would itself not have an improved capacity for survival or reproduction.[46] It would not be called "alive". For this to happen, an RNA-coded activity is needed that imparts an advantage in survival, growth, or replication for the membrane component providing internal control of cell division.[46] A ribozyme that synthesizes amphiphilic lipids and thus enables the membrane to grow would serve as an example. The membrane and the genome would then be coupled, and the "organism" as a whole could evolve, as vesicles with improved ribozymes would have a growth and replication advantage.[46] Advanced studies indeed showed that an innervesicular amplifying RNA system could cause a vesicle to grow by implementing amphiphiles from neighboring vesicles with lower osmotic pressure.[50]

6. Summary and Outlook: From Amphiphiles to Living Cells

Endogenous Fischer–Tropsch syntheses in the aqueous phase and exogenous delivery by meteorites and comets are potentially important sources of prebiotic and biogenic molecules to the early Earth. Both processes provide amphiphilic molecules that, under well-defined physicochemical conditions, assemble into membrane vesicles. Vesicles are assumed to have harbored potential prebiotic catalysis. With compartmentalization, the encapsulated replicase component is not only capable of, but also inevitably subject to, variation, natural selection, and thus Darwinian evolution.[46] On the basis of experimental studies carried out in the laboratory, we can assume that cell-like membranous compartments composed of bilayers appeared wherever organic compounds became concentrated. Additional molecules were trapped within these compartments. Life—which combines metabolism, growth, reproduction, and adaptation through natural selection—began when one or more of the components found a way not only to grow but also to reproduce by incorporating a cycle involving catalytic functions and genetic information. The key point in all attempts towards an experimental simulation of the origin of the hypothetical precursor of the first living systems is thus the link between template copying and metabolism to membrane growth and reproduction of the compartment.[82] Lipid vesicles may have served as a physical container that housed informational polymers, such as DNA and RNA, and as a metabolic system that chemically regulates and regenerates cellular components.[43]

Some authors have suggested that a lipid world may have preceded an RNA world.[1] Nonetheless, at some point in prebiotic evolution, aggregates of lipid-like molecules likely began to incorporate monomers of present-day life, such as nucleotides and amino acids. After oligomerization, catalysis and templating capacities would be enhanced within the aggregates.

An important goal for future research on the origin of life will be to systematically explore the physicochemical parameters under which cell-like vesicles could constitute a suitable microenvironment in which diverse chemical reactions could occur. These reactions include rudimentary photosynthesis, as well as the generation of RNA and protein monomers, followed by the synthesis of templating molecules in the interior space of vesicles.[1] In this context, it is widely believed that the design of an artificial cell, namely a highly simplified version of a biological cell, might be achievable in the near future[4,83] as an imaginable goal.[46] If these predictions are right, we should be hearing about some dramatic findings very soon. The question of the most likely early technological applications of artificial cell research remains as yet unanswered. In time, research will eventually produce dramatic new technologies, such as self-repairing and self-replicating nanomachines. With metabolisms and genetics unlike those of existing organisms, such machines would form the basis for a living technology possessing powerful capabilities and raising important social and ethical implications.[43] Experimentally, the potential exists to supply a population of cells with random RNA sequences to observe and determine what new ribozyme activities were most accessible and advantageous for evolving simple cells.[46] In the long run, it might even be possible to observe at least some aspects of the evolution of protein synthesis, possibly with different sets of amino acids.[46]

This work was supported by the Agence Nationale de la Recherche ANR-07-BLAN-0293 and the NASA Astrobiology Institute and Goddard Center for Astrobiology. The fluorescence microscope images of simulated precometary ices were taken in collaboration with Dr. Marla Moore, GSFC. The cover picture and 3D video were created by Adil Boujibar from Ingemedia, Toulon, France. We thank David Deamer for providing Figure 4.

Received: October 29, 2009
Revised: November 27, 2009
Published online: April 30, 2010

[1] D. Segré, D. Ben-Eli, D. W. Deamer, D. Lancet, *Origins Life Evol. Biosphere* **2001**, *31*, 119 – 145.
[2] P. Walde, *Origins Life Evol. Biosphere* **2006**, *36*, 109 – 150.
[3] F. M. Menger, A. V. Peresypkin, *J. Am. Chem. Soc.* **2001**, *123*, 5614 – 5615.
[4] H. H. Zepik, P. Walde, T. Ishikawa, *Angew. Chem.* **2008**, *120*, 1343 – 1345; *Angew. Chem. Int. Ed.* **2008**, *47*, 1323 – 1325.
[5] T. M. McCollom, G. Ritter, B. R. Simoneit, *Origins Life Evol. Biosphere* **1999**, *29*, 153 – 156.
[6] A. I. Rushdi, B. R. T. Simoneit, *Origins Life Evol. Biosphere* **2001**, *31*, 103 – 118.
[7] R. M. Hazen, D. W. Deamer, *Origins Life Evol. Biosphere* **2007**, *37*, 143 – 152.
[8] A. I. Rushdi, B. R. T. Simoneit, *Origins Life Evol. Biosphere* **2006**, *36*, 93 – 108.
[9] S. L. Miller, A. Lazcano, *J. Mol. Evol.* **1995**, *41*, 689 – 692.
[10] D. W. Deamer, J. P. Dworkin, S. A. Sandford, M. P. Bernstein, L. J. Allamandola, *Astrobiology* **2002**, *2*, 371 – 381.
[11] M. P. Bernstein, J. P. Dworkin, S. A. Sandford, G. W. Cooper, L. J. Allamandola, *Nature* **2002**, *416*, 401 – 403.
[12] G. M. Muñoz Caro, U. J. Meierhenrich, W. A. Schutte, B. Barbier, A. Arcones Segovia, H. Rosenbauer, W. H.-P. Thiemann, A. Brack, J. M. Greenberg, *Nature* **2002**, *416*, 403 – 406.
[13] U. J. Meierhenrich, *Amino Acids and the Asymmetry of Life*, Springer, Heidelberg, **2008**.

[14] U. J. Meierhenrich, G. M. Muñoz Caro, W. A. Schutte, W. H.-P. Thiemann, B. Barbier, A. Brack, *Chem. Eur. J.* **2005**, *11*, 4895 – 4900.
[15] J. P. Dworkin, D. W. Deamer, S. A. Sandford, L. J. Allamandola, *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 815 – 819.
[16] D. W. Deamer, R. M. Pashley, *Origins Life Evol. Biosphere* **1989**, *19*, 21 – 38.
[17] L. J. Allamandola, S. A. Sandford, G. Valero, *Icarus* **1988**, *76*, 225 – 252.
[18] J. R. Cronin, S. Pizzarello, *Science* **1997**, *275*, 951 – 955.
[19] S. Pizzarello, Y. Huang, M. R. Alexandre, *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 3700 – 3704.
[20] D. P. Glavin, J. P. Dworkin, *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 5487 – 5492.
[21] U. J. Meierhenrich, G. M. Muñoz Caro, J. H. Bredehöft, E. K. Jessberger, W. H.-P. Thiemann, *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 9182 – 9186.
[22] P. G. Stoks, A. W. Schwartz, *Nature* **1979**, *282*, 709 – 710.
[23] Z. Martins, O. Botta, M. Fogele, M. A. Sephton, D. P. Glavin, J. S. Watson, J. P. Dworkin, A. W. Schwartz, P. Ehrenfreund, *Earth Planet. Sci. Lett.* **2008**, *270*, 130 – 136.
[24] S. Pizzarello, Y. Huang, L. Becker, R. J. Poreda, R. A. Niemann, G. Cooper, M. Williams, *Science* **2001**, *293*, 2236 – 2239; S. Pizzarello, Y. Huang, *Meteorit. Planet. Sci.* **2002**, *37*, 687 – 696.
[25] D. W. Deamer, G. L. Barchfeld, *J. Mol. Evol.* **1982**, *18*, 203 – 206.
[26] Y. Huang, Y. Wang, M. R. Alexandre, T. Lee, C. Rose-Petruck, M. Fuller, S. Pizzarello, *Geochim. Cosmochim. Acta* **2005**, *69*, 1073 – 1084.
[27] G. Yuen, K. A. Kvenvolden, *Nature* **1973**, *246*, 301 – 302.
[28] T. Namani, D. W. Deamer, *Origins Life Evol. Biosphere* **2008**, *38*, 329 – 341.
[29] M. S. Nikolic, C. Olsson, A. Salcher, A. Kornowski, A. Rank, R. Schubert, A. Frömsdorf , H. Weller, S. Förster, *Angew. Chem.* **2009**, *121*, 2790 – 2792; *Angew. Chem. Int. Ed.* **2009**, *48*, 2752 – 2754.
[30] L. Carbone, L. Manna, C. Sönnichsen, *Angew. Chem.* **2009**, *121*, 4346 – 4347; *Angew. Chem. Int. Ed.* **2009**, *48*, 4282 – 4283.
[31] S. Rasi, F. Mavelli, P. L. Luisi, *J. Phys. Chem. B* **2003**, *107*, 14068 – 14076.
[32] C. L. Apel, D. W. Deamer, M. N. Mautner, *Biochim. Biophys. Acta Biomembr.* **2002**, *1559*, 1 – 9.
[33] T. Namani, P. Walde, *Langmuir* **2005**, *21*, 6210 – 6219.
[34] Z. Cheng, P. L. Luisi, *J. Phys. Chem. B* **2003**, *107*, 10940 – 10945.
[35] N. Berclaz, M. Müller, P. Walde, P. L. Luisi, *J. Phys. Chem. B* **2001**, *105*, 1056 – 1064.
[36] F. M. Menger, K. D. Gabrielson, *Angew. Chem.* **1995**, *107*, 2260 – 2278; *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 2091 – 2106.
[37] D. G. Hunter, B. J. Frisken, *Biophys. J.* **1998**, *74*, 2996 – 3002.
[38] B. J. Frisken, C. Asman, P. J. Patty, *Langmuir* **2000**, *16*, 928 – 933.
[39] A. I. Oparin, K. L. Gladilin, *BioSystems* **1980**, *12*, 133 – 145.
[40] H. J. Morowitz, B. Heinz, D. W. Deamer, *Origins Life Evol. Biosphere* **1988**, *18*, 281 – 287.
[41] W. F. Doolittle, J. R. Brown, *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 6721 – 6728.
[42] C. Woese, *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 6854 – 6859.
[43] S. Rasmussen, L. Chen, D. W. Deamer, D. C. Krakauer, N. H. Packard, P. F. Stadler, M. A. Bedau, *Science* **2004**, *303*, 963 – 965.
[44] K. Fontell, L. Mandell, *Colloid Polym. Sci.* **1993**, *271*, 974 – 991.
[45] P. Walde, A. Goto, P.-A. Monnard, M. Wessicken, P. L. Luisi, *J. Am. Chem. Soc.* **1994**, *116*, 7541 – 7547.
[46] J. W. Szostak, D. P. Bartel, P. L. Luisi, *Nature* **2001**, *409*, 387 – 390.
[47] M. Meot-Ner (Mautner), D. E. Elmore, S. Scheiner, *J. Am. Chem. Soc.* **1999**, *121*, 7625 – 7635.
[48] I. A. Chen, J. W. Szostak, *Biophys. J.* **2004**, *87*, 988 – 998.
[49] P.-A. Monnard, C. L. Apel, A. Kanavarioti, D. Deamer, *Astrobiology* **2002**, *2*, 139 – 152.
[50] I. A. Chen, K. Salehi-Ashtiani, J. W. Szostak, *J. Am. Chem. Soc.* **2005**, *127*, 13213 – 13219.
[51] M. M. Hanczyc, S. M. Fujikawa, J. W. Szostak, *Science* **2003**, *302*, 618 – 622.
[52] P. Walde, R. Wick, M. Fresta, A. Mangone, P. L. Luisi, *J. Am. Chem. Soc.* **1994**, *116*, 11649 – 11654.
[53] R. Wick, P. Walde, P. L. Luisi, *J. Am. Chem. Soc.* **1995**, *117*, 1435 – 1436.
[54] S. U. Egelhaaf, P. Schurtenberger, *Phys. Rev. Lett.* **1999**, *82*, 2804 – 2807.
[55] F. M. Konikoff, D. Danino, D. Weihs, M. Rubin, Y. Talmon, *Hepatology* **2000**, *31*, 261 – 268.
[56] D. D. Lasic, R. Joannic, B. C. Keller, P. M. Frederik, L. Auvray, *Adv. Colloid Interface Sci.* **2001**, *89–90*, 337 – 349.
[57] M. A. Long, E. W. Kaler, S. P. Lee, *Biophys. J.* **1994**, *67*, 1733 – 1742.
[58] I. Grillo, E. I. Kats, A. R. Muratov, *Langmuir* **2003**, *19*, 4573 – 4581.
[59] P. K. Vinson, Y. Talmon, A. Walter, *Biophys. J.* **1989**, *56*, 669 – 681.
[60] M. M. Hanczyc, S. S. Mansy, J. W. Szostak, *Origins Life Evol. Biosphere* **2007**, *37*, 67 – 82.
[61] I. A. Chen, R. W. Roberts, J. W. Szostak, *Science* **2004**, *305*, 1474 – 1476.
[62] D. K. Kondepudi, I. Prigogine, *Modern Thermodynamics*, Wiley, New York, **1998**.
[63] S. S. Mansy, J. W. Szostak, *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 13351 – 13355.
[64] S. F. Mansy, J. P. Schrum, M. Krishnamurthy, S. Tobé, D. A. Treco, J. W. Szostak, *Nature* **2008**, *454*, 122 – 125.
[65] S. G. Martin, M. Berthelot-Grosjean, *Nature* **2009**, *459*, 852 – 856.
[66] J. B. Moseley, A. Mayeux, A. Paoletti, P. Nurse, *Nature* **2009**, *459*, 857 – 860.
[67] D. V. Volodkin, A. G. Skirtach, H. Möhwald, *Angew. Chem.* **2009**, *121*, 1839 – 1841; *Angew. Chem. Int. Ed.* **2009**, *48*, 1807 – 1809.
[68] E. Blöchliger, M. Blocher, P. Walde, P. L. Luisi, *J. Phys. Chem. B* **1998**, *102*, 10383 – 10390.
[69] S. Lonchin, P. L. Luisi, P. Walde, B. H. Robinson, *J. Phys. Chem. B* **1999**, *103*, 10910 – 10916.
[70] S. Chungcharoenwattana, H. Kashiwagi, M. Ueno, *Colloid Polym. Sci.* **2005**, *283*, 1180 – 1189.
[71] D. P. Summers, J. Noveron, R. C. B. Basa, *Origins Life Evol. Biosphere* **2009**, *39*, 127 – 140.
[72] A. C. Chakrabarti, D. W. Deamer, *J. Mol. Evol.* **1994**, *39*, 1 – 5.
[73] P.-A. Monnard, D. W. Deamer, *Origins Life Evol. Biosphere* **2001**, *31*, 147 – 155.
[74] S. Paula, A. G. Volkov, A. N. van Hoek, T. H. Haines, D. W. Deamer, *Biophys. J.* **1996**, *70*, 339 – 348.
[75] J. F. Nagle, H. L. Scott, *Biochim. Biophys. Acta Biomembr.* **1978**, *513*, 236 – 243.
[76] J. A. Hamilton, *J. Lipid Res.* **1998**, *39*, 467 – 481.
[77] I. Chen, J. W. Szostak, *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 7965 – 7970; S. S. Mansy, *Int. J. Mol. Sci.* **2009**, *10*, 835 – 843.
[78] D. W. Deamer, *Nature* **2008**, *454*, 37 – 38.
[79] R. Furuuchi, E.-I. Imai, H. Honda, K. Hatori, K. Matsuno, *Origins Life Evol. Biosphere* **2005**, *35*, 333 – 343.
[80] T. Oberholzer, R. Wick, P. L. Luisi, C. K. Biebricher, *Biochem. Biophys. Res. Commun.* **1995**, *207*, 250 – 257.
[81] More detailed information on many possible approaches to evolving and designing an RNA replicase is given in Ref. [46].
[82] H. H. Zepik, Walde P, *ChemBioChem* **2008**, *9*, 2771 – 2772.
[83] P.-A. Monnard, H.-J. Ziock, *Origins Life Evol. Biosphere* **2007**, *37*, 469 – 472.

