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SURFACTANT ASSEMBLIES AND THEIR VARIOUS POSSIBLE ROLES FOR THE ORIGIN(S) OF LIFE

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Abstract. A large number of surfactants (surface active molecules) are chemically simple compounds that can be obtained by simple chemical reactions, in some cases even under presumably prebiotic conditions. Surfactant assemblies are self-organized polymolecular aggregates of surfactants, in the simplest case micelles, vesicles, hexagonal and cubic phases. It may be that these different types of surfactant assemblies have played various, so-far underestimated important roles in the processes that led to the formation of the first living systems.

Although nucleic acids are key players in the formation of cells as we know them today (RNA world hypothesis), it is still unclear how RNA could have been formed under prebiotic conditions. Surfactants with their self-organizing properties may have assisted, controlled and compartimentalized some of the chemical reactions that eventually led to the formation of molecules like RNA. Therefore, surfactants were possibly very important in prebiotic times in the sense that they may have been involved in different physical and chemical processes that finally led to a transformation of non-living matter to the first cellular form(s) of life. This hypothesis is based on four main experimental observations: (i) Surfactant aggregation can lead to cell-like compartimentation (vesicles). (ii) Surfactant assemblies can provide local reaction conditions that are very different from the bulk medium, which may lead to a dramatic change in the rate of chemical reactions and to a change in reaction product distributions. (iii) The surface properties of surfactant assemblies that may be liquid- or solid-like, charged or neutral, and the elasticity and packing density of surfactant assemblies depend on the chemical structure of the surfactants, on the presence of other molecules, and on the overall environmental conditions (e. g. temperature). This wide range of surface characteristics of surfactant assemblies may allow a control of surface-bound chemical reactions not only by the charge or hydrophobicity of the surface but also by its "softness". (iv) Chiral polymolecular assemblies (helices) may form from chiral surfactants.

There are many examples that illustrate the different roles and potential roles of surfactant assemblies in different research areas outside of the field of the origin(s) of life, most importantly in investigations of contemporary living systems, in nanotechnology applications, and in the development of drug delivery systems. Concepts and ideas behind many of these applications may have relevance also in connection to the different unsolved problems in understanding the origin(s) of life.

Keywords: Micelles, vesicles, bilayers, liposomes, surfactants, protocell, micellar catalysis, surfaces

Abbreviations: Amino acids without specification of the configuration are L-amino acids; H-ONp, p-nitrophenol; CTAB, cetyltrimethylammonium bromide (= hexadecyltrimethylammonium bromide); CTAOH, cetyltrimethylammonium hydroxide (= hexadecyltrimethylammonium hydroxide); CTAT, cetyltrimethylammonium tosylate (= hexadecyltrimethylammonium tosylate); SOS, sodium octylsulfate; SDS, sodium dodecylsulfate; SDBS, sodium dodecylbenzenesulfonate; $C_{10}E_4$, tetraethyleneglycoldecyl ether (= n-$C_{10}H_{21}(OCH_2CH_2)_3OCH_2CH_2OH$);

$C_{12}E_8$, octaethyleneglycoldodecyl ether (= n-$C_8H_{17}(OCH_2CH_2)_7OCH_2CH_2OH$); DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; DPPA, 1,2-dipalmitoyl-*sn*-glycero-3-phosphate; DSPC, 1,2-distearoyl-*sn*-glycero-3-phosphocholine; DOPC, 1,2-dioleoyl-*sn*-glycero-3-phosphocholine; DOPG, 1,2-dioleoyl-*sn*-glycero-3-phosphoglycerol; POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine, POPG, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoglycerol; DMUPC, 1,2-dimyristoyluridinophosphocholine; DPUPC, 1,2-dipalmitoyluridinophosphocholine; DOPE, 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine; DMP-Cyt, 1,2-dimyristoyl-*sn*-glycero-3-phosphocytidine; PC, phosphatidylcholine; EEDQ, 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline.

Introduction

Despite the many excellent origin(s)-of-life-books that have been written, for example (Oparin, 1938; Morowitz, 1992; de Duve, 1995; Zubay, 1996; Dyson, 1999; Lahav, 1999; Maynard Smith and Szathmáry, 1999; Wills and Bada, 2000; Fry, 2000; Schopf, 2002), the only thing which is for sure in all the research on the origin (or origins) of life is that nobody knows how life originated. There are a lot of facts and speculations in this field (Orgel, 1998). Due to the complexity of even simple unicellular organisms, it is rather difficult to imagine how a living system could have originated from the non-living.

A popular view on the early events in the origin of life is the so-called "RNA world scenario" (Gilbert, 1986) in which RNA molecules are believed to have played an essential role as information carrying, possibly self-replicating and possibly catalytically active molecules (Doudna and Cech, 2002). Although it is currently unclear how RNA or oligoribonucleotides or even single nucleotides (Orgel, 2003) could have been formed under prebiotic conditions, the "RNA world-", "pre-RNA world-" (Joyce *et al*., 1987; Nielson, 1993) or "proto-RNA world scenario" (Anet, 2004) currently dominates the scientific origin of life discussions among chemists and molecular biologists.

The aim of the present paper is to draw attention to the possible roles of surfactant assemblies during prebiological chemical evolution. It is not only that surfactants may form closed, bilayered, cell-mimicking compartments (vesicles) - which undoubtedly underlines the important role of these amphiphilic molecules in the formation of the first cell(s) – the spontaneous self-organization of surfactants into a variety of different types of aggregates with their corresponding physicochemical properties deserves a discussion of other possible prebiotic roles of these self-organized molecular assemblies. All these considerations are related to the hypothesis of the "lipid world" scenario (Segré *et al*., 2001). The "lipid world" scenario assumes not only that amphiphilic boundary structures (lipids) contributed to the emergence of life at large, but that lipids played important roles in a *very early* evolutionary step in the emergence of life on Earth (Segré *et al*., 2001). It is evident that surfactants (amphiphiles) were among the molecules prebiotically formed.

In the first section of this article, a short summary of some of the current lines of thinking about the origin(s) of life, and a few simple considerations concerning the first cells are described. The main part of the paper deals with surfactant self-assemblies and their possible relevance for the origin(s) of life.

Some of the Origin-of-Life-Related Questions and Their Relation to Surfactants

How simple and how small are the simplest known living organisms? Even presumably simple unicellular organisms like Mycoplasmas are extremely complex, as all cells are. The simplest known cells have less than 500 but more than 200 essential genes and it is likely that a viable cell living in today's environment needs probably not less than 200–300 genes (Mushegian, 1999). The minimum cell size enabling independent contemporary life is considered to be in the range of 140 nm to 300 nm (Velimirov, 2001). Without membrane-forming *surfactants* (lipids) that separate the cell's interior from the environment, there would be no cellular life as we know it today.

Which types of molecules are of potential prebiotic relevance? In laboratory experiments carried out under presumably prebiotic conditions, a large number of different simple organic molecules have been synthesized in the past (Miller, 1998), including glycine, simple peptides, formic acid, acetic acid, propionic acid, urea, adenine, cytosine, uracil, fatty acids and other *surfactants* (see Table I). Some of these types of molecules – including surfactants like fatty acids (Deamer, 1985; Naraoka *et al*., 1999) – were apparently brought to Earth in prebiotic times by carbon-rich meterorites (Sephton, 2002) or by interplanetary dust particles (Flynn *et al*., 2004).

Despite the uncertainty about the site where and about the conditions under which the first living organisms originated, one can claim that simple organic molecules, including simple surfactants, can be obtained under presumably prebiotic conditions, at least as long as the molecules are chemically relatively simple, and as long as they are not optically pure. With respect to the last point, the origin of biomolecular homochirality is certainly an important issue to address, although it does not seem to be *the* central problem in understanding the origin of life. In most of the arguments about the origin of homochirality, a step for the amplification of the enantiomer in excess is necessary, e. g. by asymmetric autocatalytic processes. A small enantiomeric excess obtained through stochastic symmetry breaking processes (e. g. Bolli *et al*., 1997) may be enough to obtain after several reaction cycles a product with much higher optical purity (Bonner, 1999; Podlech, 2001; Soai *et al*., 2004). *Surfactants* may be involved in autocatalytic processes, e. g. (Bachmann *et al.,* 1992; Morigaki *et al*., 1997; Buijnsters *et al.,* 2001), in some cases leading to an amplification of enantioselective reactions (Scrimin *et al.,* 1994; Bertoncin *et al.,* 1998).

TABLE I

Some of the surfactants that are of potentially prebiotic relevance

Some of the surfactants that have been synthesized in the laboratory under presumably prebiotic conditions, see also (Oró, 1994; Simoneit, 2004)	
Linear fatty acids (C_n stands for $C_{n-1}H_{2n-1}COOH$):	
C_5–C_{20}	Nooner *et al*., 1979
C_6–C_{22}	McCollom *et al*., 1999
C_7–C_{22}	Rushdi and Simoneit, 2001
Linear fatty alcohols (C_n stands for $C_nH_{2n+1}OH$):	
C_6–C_{23}	McCollom *et al*., 1999
C_8–C_{32}	Rushdi and Simoneit, 2001
Monoacylglycerols	Hargreaves *et al*., 1977; Apel and Deamer, 2005
Phospholipids:	
Different types	Hargreaves *et al*., 1977
Phosphatidic acids	Epps *et al*., 1978
Phosphatidylcholines	Rao *et al*., 1982
Phosphatidylethanolamines	Rao *et al*., 1987
Examples of surfactants that can be considered as prebiotically relevant	
Polyprenylphosphates and polyprenylpyrophosphates	Ourisson and Nakatani, 1994; Pozzi *et al*., 1996; Ariga *et al*., 2005
Mono-*n*-alkylphosphates	Walde *et al*., 1997
Fatty acids that have been isolated from carbonaceous meteorites, see also (Sephton, 2002).	
Linear fatty Acids (C_n stands for $C_{n-1}H_{2n-1}COOH$):	
C_8–C_{12}	Shimoyama *et al*., 1989
C_9	Deamer, 1997

Under which environmental conditions did the first living organisms originate? It may be that "primitive" life originated somewhere in the universe and that corresponding "primitive" living systems were brought onto the Earth (Raulin-Cerceau, 1998), although it is generally assumed that life on Earth has its origin on Earth. There are different hypotheses about the possible sites for the origin of the first life forms on Earth (Hiscox, 2001), for example (i) close to submarine hydrothermal vents (Corliss *et al*., 1981), at the bottom of the ocean, where magma (liquid rock) spills through the Earth's crust and reacts with the seawater (Furnes *et al*., 2004); (ii) on minerals, such as clays (Cairns-Smith, 1985), pyrite (FeS_2, Wächtershäuser, 1992; Edwards, 1998; Tributsch *et al*., 2003), or particular aluminium-substituted silica (Smith *et al*., 1999); (iii) in a primitive ocean, in a primordial "soup" of diluted nutrient organic compounds (Oparin, 1938).

In contrast to thermolabile, free RNA, *surfactants* may be stable under extreme conditions of temperature and pH, it just depends on the chemical structure of the surfactants. Therefore, there is no general surfactant stability limitation with respect to the possible sites for the origin of the first cells. Surfactants may accumulate on

surfaces and they may self-assemble in a dilute solution, in both cases leading to high local surfactant concentrations, just as required for the formation of the first units of a living system.

What was the appearance of the first living cellular organisms and their precursor structures? It is reasonable to assume that precursor structures of the first cell(s), so-called "progenotes" (Woese, 1998; Doolittle and Brown, 1994) or "protocells" (Morowitz *et al*., 1988), "protobionts" (Oparin, 1965) or "probionts" (Oparin and Gladilin, 1980), were a bit simpler than the first cells. They may have been composed of simple membranes (as boundaries of the structures), an internal set of catalysts (possibly simple oligopeptides/proteins and/or metal ion complexes), sequence-encoded information-carrying molecules (simple RNA/DNA), and a polypeptide/protein synthesis apparatus (possibly a mixture of RNAs and proteins; or just RNAs (Moore and Steitz, 2002)). The membrane – inorganic (Russell and Hall, 1997) or organic based on *surfactants* (Deamer *et al.,* 2002; Ourisson and Nakatani, 1994) – must have been permeable for certain molecules (food and waste) but not for others (those involved in establishing metabolic network reactions). A schematic drawing of such a hypothetical precursor structure is shown in Figure 1. If this structure is considered as reasonable model, a number of complex chemical

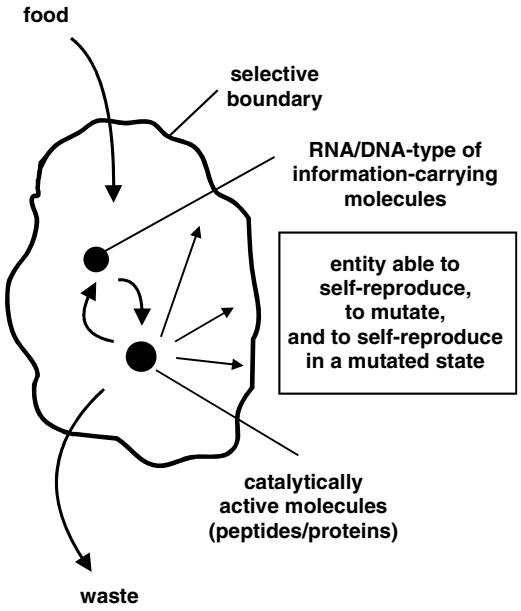


Figure 1. Very schematic drawing of a hypothetical precursor structure of the first cell(s), see also the various discussions about protocellular structures and the universal minimal cell (e. g. Margulis, 1993; Segré and Lancet, 2000; Luisi, 2002a; Trevors, 2003; Martin and Russell, 2002). A theoretical description of a minimal cell that includes three autocatalytic subsystems – metabolism, template copying and boundary growth – is known as "chemoton" (Gánti, 2003).

compounds (particularly specific RNA/DNA molecules and proteins) need to have evolved from simple molecules. Furthermore, in addition to polymerization, replication of RNA/DNA was needed in order to allow a chemical evolution of the molecularly simple precursor structures to the more complex first cell(s).

Nobody currently knows how to prepare convincing hypothetical precursor structures of the first cells, or how such structures and the contents of these structures may have been formed on the early Earth (or somewhere else). It is possible that a coevolution of nucleic acids, proteins, cofactors, and metabolic chemical systems with autocatalytic feedback mechanisms within – or associated to – compartments occurred (Segré *et al*., 2001; Szostak *et al*., 2001; Rasmussen *et al*., 2004). *Surfactants* most likely played a major role in these processes, as they were probably important molecules of at least the membranes of the protocells. Taking into account that metabolism and replication are essential features of contemporary life, it may also well be that either (i) metabolism evolved before replication, or (ii) replication came before metabolism. There are in deed different arguments for a "metabolism-first" theory, e. g. (Wächtershäuser, 1997; Dyson, 1999; Anet, 2004), as well as for a "genetics or replication-first" theory, e. g. (Gilbert, 1986; Orgel, 2000).

General Aggregation Behavior of Surfactants

Chemically simple single-chain surfactants

In the case of chemically simple *single-chain surfactants* such as sodium octanoate, sodium dodecyl sulfate (SDS) or $C_{12}E_8$ (octaethyleneglycoldodecyl ether), see Figure 2, the aggregates formed in (salt-free) water are almost perfectly spherical micelles (Figure 3). Depending on the chemical structure of the surfactants, and depending on the composition of the aqueous phase, the micelles can also be non-spherical (disk- or rod-like). Single-chain surfactants, however, do not only self-aggregate into conventional micelles. Depending on the surfactant (e. g. the case of CTAB), a complex self-aggregation behavior – including the formation of branched aggregates and network structures – occurs by just varying the surfactant concentration or by changing the salt content (Lequeux and Candau, 1994). On increasing the concentration of a conventional, simple single-chain surfactant above the critical concentration for micelle formation (cmc), more micelles are first formed, and then the size of the micelles increases, in parallel with a change in the micelle's shape, until a transformation into non-micellar phases occurs.

So-called cubic phases form in surfactant/water systems at high surfactant concentrations. One extensively studied example is monoolein, a chemically simple single-chain surfactant (Larsson, 1989; Lindblom and Rilfors, 1989). Like in the case of micelles, the hydrocarbon region and the head-group area of cubic phases are liquid-like (non-crystalline). If one further increases the complexity of the system

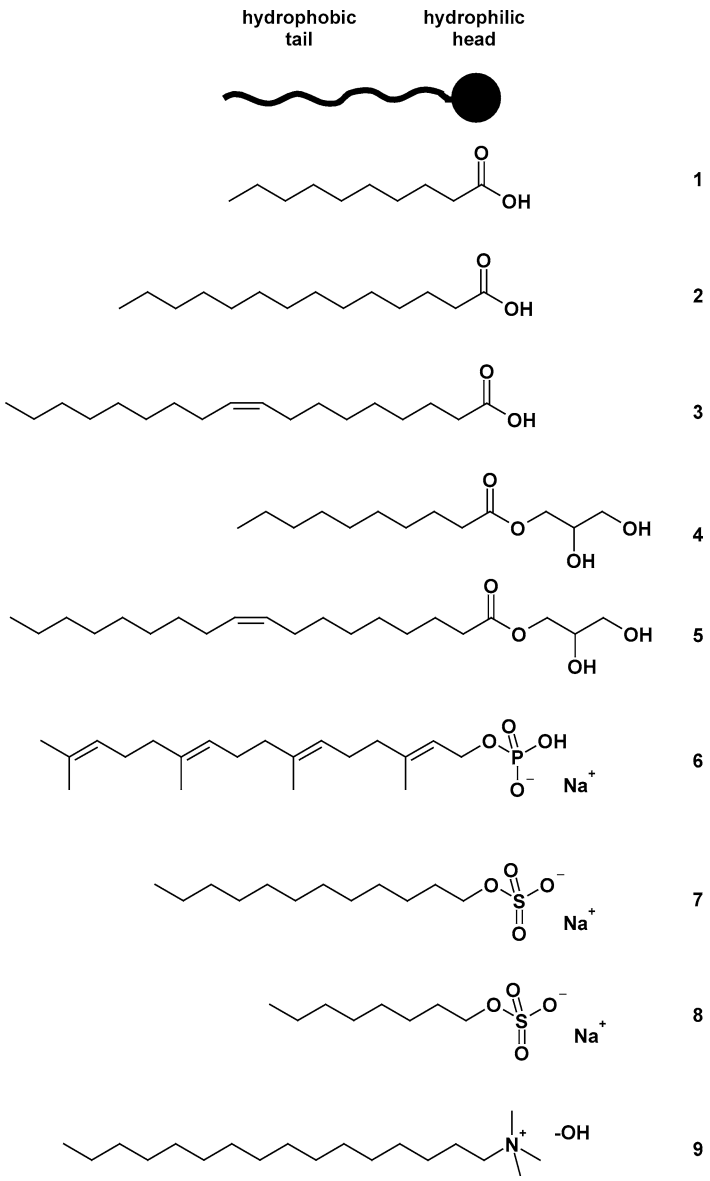


Figure 2. Schematic representation and examples of conventional *single*-chain surfactants. **1**: decanoic acid; **2**: myristic acid (tetradecanoic acid); **3**: oleic acid (*cis*-9-octadecenoic acid); **4**: *rac*-1-decanoylglycerol (1-monodecanoyl-*rac*-glycerol); **5**: *rac*-1-oleoylglycerol (1-monoolein, monoolein, 1-monooleoyl-*rac*-glycerol); **6**: sodium geranylgeraniolphosphate; **7**: SDS (sodium dodecylsulfate); **8**: SOS (sodium octylsulfate); **9**: CTAOH (cetyltrimethylammonium hydroxide). **1** has been synthesized under presumably prebiotic conditions and it has been extracted from carbonaceous meteorites. Polyprenyl phosphates like **6** are considered as prebiotically relevant single-chain surfactants (see Table I).

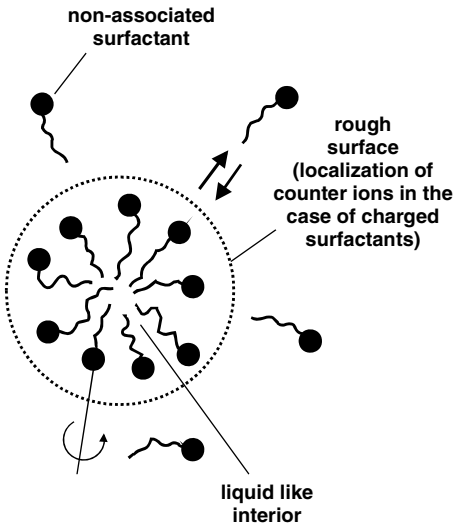


Figure 3. Schematic representation of a part of a micellar solution just above the critical concentration for micelle formation (cmc), showing a cross-section through a spherical micelle and showing non-associated surfactant molecules which are in dynamic equilibrium with the micelles. The concentration of non-associated surfactant is about the cmc, in the case of sodium decanoate as example about 50 mM (Namani and Walde, 2005). The entire micelle is in a fluid state with a liquid-like interior and a fluid surface, caused by rapid molecular motions.

by adding just one simple component more, an organic solvent, one arrives at a so-called three-component system composed of a surfactant, water, and an organic solvent, and – depending on the composition -one obtains a lamellar phase, hexagonal and cubic phases, as well as oil-in-water and water-in-oil microemulsions (inverted or reversed micelles).

The phase behavior of a system containing *more than one surfactant* is complex, as illustrated with the case where two chemically different types of surfactants are mixed with water, H_2O/surfactant X/surfactant Y. This simple system can lead to aggregates that only form since the two amphiphiles synergistically "cooperate". They show a different aggregation behavior if compared with their individual self-assembly properties. The reason for this is the likely non-homogeneous distribution of the surfactants within the mixed aggregate. If for example certain aqueous mixtures of a cationic surfactant (=surfactant X) and an anionic surfactant (=surfactant Y) are analyzed, it is found that dispersed uniform, unilamellar "catanionic vesicles" are rapidly formed without any input of external energy (Kaler *et al*., 1989; Lasic *et al*., m 2001; Jung *et al*., 2001). The true equilibrium state seems to be homogeneous, *unilamellar* vesicles, in clear contrast to the micelle formation observed if only one type of surfactant is used. Examples for the "spontaneous" formation of unilamellar vesicles include H_2O/CTAB/SOS (Yatcilla *et al*., 1996), or

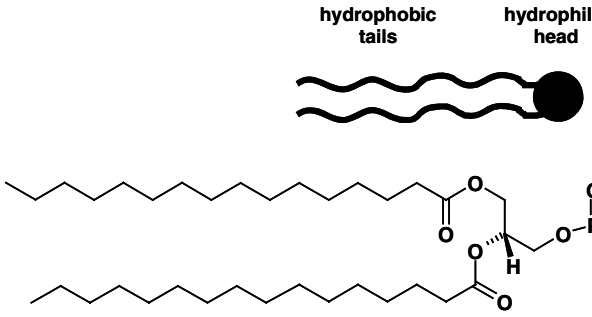
H_2O/CTAT/SDBS (Kaler *et al*., 1992). Simple H_2O/surfactant X/surfactant Y mixtures can also lead to the formation of unexpectedly complex geometric structures (Dubois *et al*., 2004). In the case of H_2O/CTAOH (hexadecyltrimethylammonium hydroxide)/myristic acid, disks (Zemb *et al*., 1999) or icosahaeders (Dubois *et al*., 2001) form, depending on the composition and on the temperature. In this case, the temperature has to be below the chain melting temperature of one of the two amphiphiles (myristic acid), and above the chain melting temperature of the other amphiphile (CTAOH), allowing the existence of non-homogeneously mixed two-dimensional solid as well as liquid domains (molecular segregation).

At this stage of the general description of surfactant assemblies, two points should be stressed which are important with respect to the discussion of the possible roles of surfactant aggregates during prebiotic evolution: (i) Chemically extremely simple *single-chain surfactants*, without any complex head group structures - just like those shown in Figure 2, and like the single-chain amphiphiles listed in Table I – self-assemble under thermodynamic control spontaneously into a variety of different surfactant aggregates. (ii) The type of aggregate and the properties of the aggregate formed are entirely controlled by the chemical structure(s) of the surfactant(s) and by the conditions under which the surfactant self-assembly process occurs (salt content, temperature). If one even considers local conditions where transient concentration or temperature gradients exist, different types of metastable aggregates may coexist within a narrow space. (iii) The complexity of the surfactant aggregate may increase with an increase in the number of surfactant components, even combining crystalline as well as liquid ("soft") domains within the same aggregate.

Conventional double-chain surfactants

The chemical structures of a number of *double-chain amphiphiles* are shown in Figure 4. In comparison with a single chain surfactant, the overall hydrophobicity of a double chain amphiphile is increased, resulting in a decrease in the water-solubility, and as consequence to a self-assembly at a lower concentration than in the case of the corresponding single chain surfactant. Furthermore, the increased volume of the hydrophobic part influences the packing of the molecules in the aggregates and the resulting aggregate type, aggregate size and shape.

Straight double-chain amphiphiles are in a first approximation cylindrical geometric objects in which the hydrophilic head group represents one end of the cylinder. Since a packing of cylinders into spherical micelles is less likely than packing the cylinders into bilayers, it is understood why double chain amphiphiles like DPPC (1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine) in dilute water do not self-assemble into micelles but preferentially form bilayers over a wide range of concentration. In dilute aqueous solutions, the bilayers are present as dispersed, self-closed spherical vesicles, generally heterogeneous with respect to size and lamellarity. The concentration of non-associated DPPC is usually not considered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hydrophilic head

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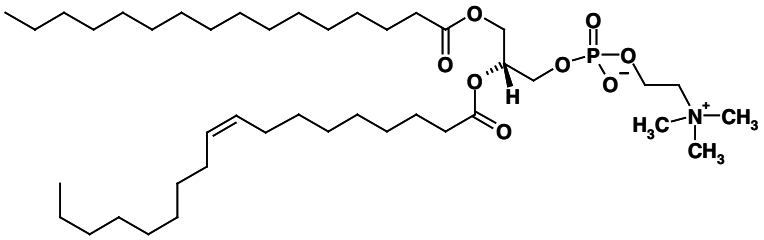
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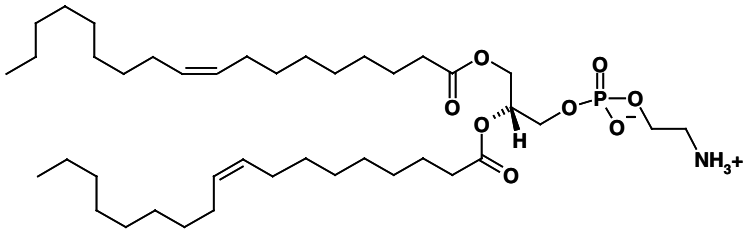
11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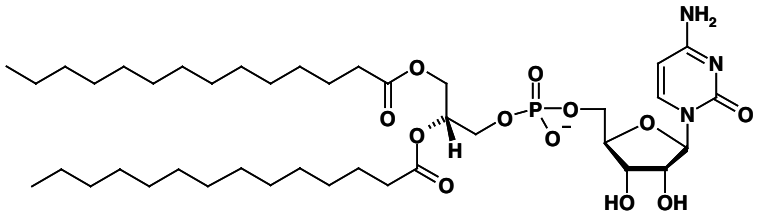
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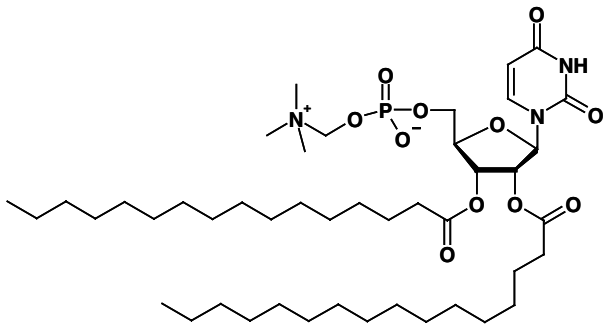
13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14

since it is as low as about 0.1 nM (Smith and Tanford, 1972). The physico-chemical properties of DPPC bilayers considerably change on varying the temperature at a fixed concentration. At high temperature – above 41 °C, the main phase transition temperature (T_m), and above 20 wt% water – the molecules are in a fluid state. This fluid state is called "liquid-disordered" (l_d, Trandum *et al.*, 2000) or L_α phase. By lowering the temperature below T_m, the bilayer transforms into a rigid, "solid-ordered" state (s_o, Trandum *et al.*, 2000). The saturated hydrophobic chains are then mainly with *trans* conformations that allow a dense packing of the DPPC molecules. Since the physico-chemical properties of bilayers formed above T_m are drastically different if compared to below T_m, a modulation of the bilayer properties from a 'crystalline-like' to a 'fluid-like' state is possible by simply varying the temperature.

In addition to the lamellar phase, and similarly to *single-chain* amphiphiles, *double-chain* surfactants above T_m may also self-assemble into cubic phases in concentrated systems, and a number of examples investigated in detail are known from literature, such as DOPC (1,2-dioleoyl-*sn*-glycero-3-phosphocholine) or DOPE (1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine) (Lindblom and Rilfors, 1989).

The Formation of Vesicles as Compartments

Vesicles of different sizes and lamellarities are usually only kinetically stable if prepared under laboratory conditions, representing "meta-stable systems" that can, however, be stable for a relatively long period of time (days, weeks, months). Examples of vesicles formed from single-chain surfactants are shown in Figure 5. DPPC and POPC are typical conventional double-chain surfactants that lead to the formation of vesicles when dispersed in aqueous solution above T_m. In contrast to micelles, homogeneous *unilamellar* vesicles usually do not form "spontaneously" without the input of external mechanical (or chemical) energy (see the apparent exception of "catanionic vesicles"), see for example (Walde, 2004). Most vesicles are spherical if the osmotic pressures inside and outside of the vesicles are the same. The vesicle diameter can be as small as about 20–30 nm and as large as dozens of micrometers (Luisi and Walde, 2000). The physico-chemical properties of vesicles – such as size stability – depend on the chemical structure of the amphiphile from

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Figure 4. Schematic representation of a conventional *double*-chain surfactant and chemical structures of double-chain surfactants similar to those that have been synthesized under presumably prebiotic conditions (**10–12**, see Table I), together with nucleoside-containing surfactants (**13, 14**) of which the self-association behavior has been studied in the past. **10**: DPPA (1,2-dipalmitoyl-*sn*-glycero-3-phosphate, mono sodium salt); **11**: POPC (1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine); **12**: DOPE (1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine); **13**: DMP-Cyt (1,2-dimyristoyl-*sn*-glycero-3-phosphocytidine); **14**: DPUPC (1,2-dipalmitoyluridinophosphocholine).

which the vesicles are made, on the size of the vesicles, on the lamellarity, on the temperature and pressure, and on the composition of the aqueous solution in which the vesicles are dispersed.

The Formation of Chiral Aggregates from Chiral Surfactants

By increasing the complexity of the chemical structure of a surfactant, and by choosing an appropriate temperature, non-conventional chiral self-assembled surfactant structures may form in aqueous solution, usually as precipitate. These aggregates include dispersed helical bands and tubes that extend over several micrometers. The formation of these types of aggregates is a consequence of the chirality of the surfactant molecules and (often) a result of a molecular crystallization process

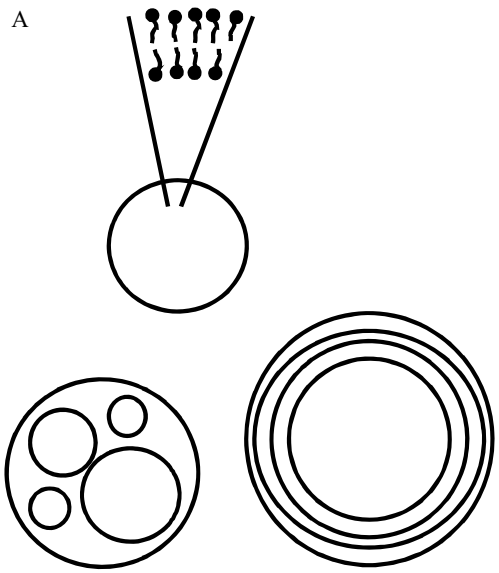
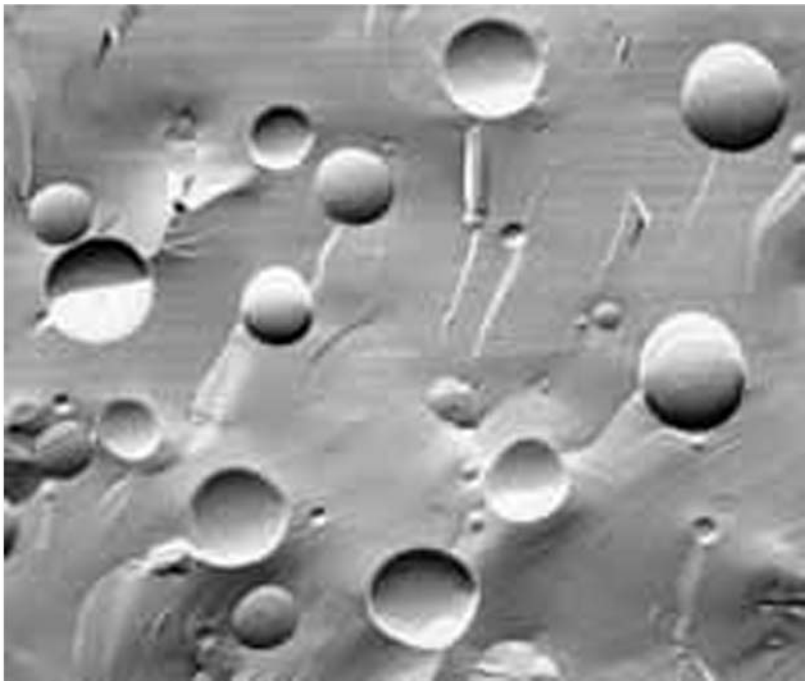


Figure 5. Vesicles composed of *single*-chain amphiphiles. **A:** Schematic representation of the cross-section through an unilamellar vesicle (top), a multilamellar vesicle (right hand side) and a multivesicular vesicle (left hand side); the diameter of a vesicle can vary from about 30 nm to more than 100 μm. The vesicle shell(s) are composed of bilayers made from single-chain surfactants that are in equilibrium with non-associated surfactants. In the case of short-chain surfactants, the concentration of non-associated molecules can be quite high (about 20 mM for decanoic acid vesicles at pH 7.0), see (Namani and Walde, 2005). **B:** Freeze fracture electron micrograph of unilamellar vesicles formed from dodecylphosphate (50 mM) at pH 2; width of the picture: 3.6 μm; see also (Walde *et al*., 1997). **C:** Light micrograph of vesicles formed from 25 mM oleic acid and 25 mM sodium oleate (pH 8.6); width of the picture: 40 μm. **D:** Light micrograph of oleic acid/oleate vesicles in 0.2 M bicine buffer, pH 8.6, stained with rhodamine B; width of the picture: 33 μm. Electron micrograph taken by Michaela Wessicken, light micrographs taken by Roger Wick, ETH-Zürich. (*Continued on next page)*

B



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D

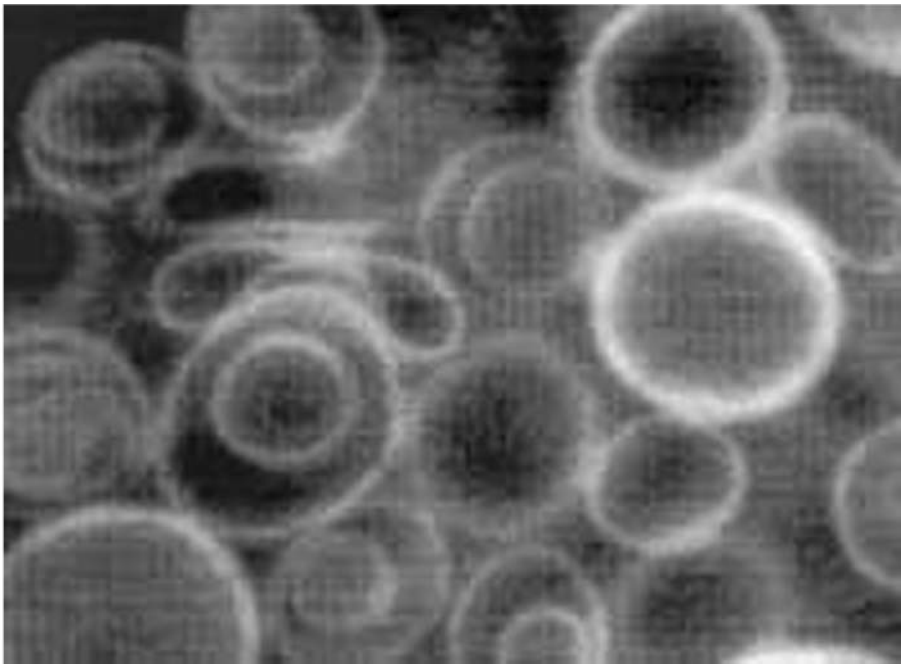


Figure 5. (*Continued*)

occurring at a temperature below T_m of the amphiphile, with a significant decrease in the polar head group mobility, often H-bond interactions, see (Fuhrhop and Helfrich, 1993; Kunitake, 1992). A few selected examples are mentioned: (i) Chiral phospholiponucleosides (like 1,2-dimyristoyl-*sn*-glycero-3-phosphocytidine, see Figure 4) in aqueous solution below T_m form helical strands (Yanagawa *et al*., 1989; Itojima *et al*., 1992), while vesicles are formed from these type of lipids above T_m (Bonaccio *et al*., 1994; Bonaccio *et al*., 1996). A comparable behavior has been observed more recently with chiral double-chain nucleolipids like DPUPC (1,2-dipalmitoyluridinophosphocholine, see Figure 4), as shown in Figure 6 (Moreau *et al*., 2004). (ii) Chiral amphiphilic amino acid derivatives in aqueous solution below T_m can form helical strands (Nakanishi *et al*., 1985; Nakashima *et al*., 1994; Cescato *et al*., 1997; Imae *et al*., 2000). (iii) Chiral N-octyl- and N-dodecyl-D-gluconamides form helical strands in aqueous solution (Köning *et al*., 1993).

It may well be that such type of crystalline-like helical aggregates played a role in prebiotic times as a kind of template for a (stereospecific) adsorption and preorganisation of reacting molecules. Some of these surfactant aggregates seem to resemble DNA (Yanagawa *et al*., 1989; Itojima *et al*., 1992; Moreau *et al*., 2004), see Figure 6.

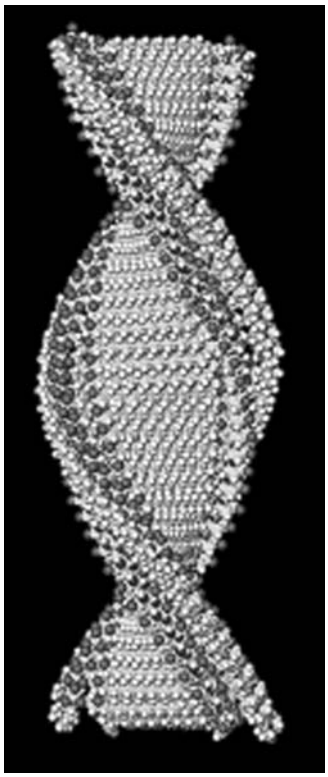


Figure 6. Schematic representation of a section of DNA-like helical fibers that are formed in aqueous solution from the chiral double-chain nucleoside phosphocholine DPUPC upon lowering the temperature below T_m. The fibres are composed of twisted bilayers. Reproduced from (Moreau *et al*., 2004) with permission from the American Chemical Society.

Possible Prebiotic Roles of Surfactant Assemblies

Surfactant assemblies may have played so far largely underestimated important roles during the chemical processes that finally led to the formation of the first cell(s). The types of surfactant assemblies considered here are not only boundary structures (Deamer, 1985) that provide a membrane to separate an internal entity from the environment, but there are also other type of aggregates to be taken into account. The possible general role of surfactant assemblies for the origin(s) of life as discussed by Luisi (Luisi, 2000) has not yet been considered seriously enough, although the general possible importance of surfactants during prebiotic times has certainly been recognized recently (Segré *et al*., 2001; Rasmussen *et al*., 2003).

First of all, surfactant aggregates have properties that are very different from the properties of the individual surfactant molecules that build the aggregates (Menger, 1991; Svetina and Žekš, 2002). One may call these properties

"emergent properties", a kind of new qualities of simple chemical systems, just like in the case of emergent properties of more complex systems (Luisi, 2002b). It may well be that through the prebiotic appearance of surfactant molecules, the self-assembly of these surfactants into aggregates eventually led to a kind of control or modification of certain chemical reactions, which may have resulted in a surfactant-assisted formation of chemically more complex systems.

It is known that surfactant aggregates may influence many different chemical processes more or less efficiently, and a number of studies have shown that surfactant aggregates (i) may significantly influence the chemical reactivity of molecules; (ii) may host molecules and protect them from the environment; (iii) may solubilize or adsorb starting materials of chemical reactions and/or reaction products; (iv) may influence the reaction product distribution; (v) may accelerate chemical reactions; (vi) may inhibit chemical reactions; (vii) may provide a template for chemical reactions; or (viii) may change the conformation equilibrium of molecules that interact with the surfactant aggregates.

In the following, a couple of selected examples from different research areas will be mentioned in order to illustrate the enormous potential of surfactant aggregates as reaction compartments and reaction media at large. The examples given are by no means a complete list. They should only show how relatively simple surfactants can have a number of remarkable effects in their self-assembled state, as a result of the properties that arise as a direct consequence of the self-organization of the surfactants.

Surfactant aggregates as reaction media

Compounds which are not soluble in an aqueous solution may be soluble in a micellar solution due to solubilization within the hydrophobic domain of micellar aggregates. In this way, intermolecular associations and chemical transformations may occur which would not take place in a micelle-free solution, or which would only occur with considerably lower reaction rates (Bunton, 1991). The micelles may act as a kind of catalyst, and the whole process is often called "micellar catalysis" (Bunton, 1991; Taşcioğlu, 1996), although it may not be true catalysis since the backward reaction is generally not catalyzed by the micelles (Fletcher and Robinson, 1984). In addition to "micellar catalysis", "micellar inhibition" is also observed: a bimolecular chemical reaction occurring easily in aqueous solution may be slowed down because one of the reaction partners is adsorbed by the micelles (Menger and Portnoy, 1967).

From the many examples described in the literature, three cases are mentioned here to demonstrate the potential of micelles (and surfactant aggregates at large) as non-aqueous nano- or micrometer-sized reaction media. Although the examples given may *not* involve currently accepted prebiotic molecules, they should only illustrate the principles and concepts.

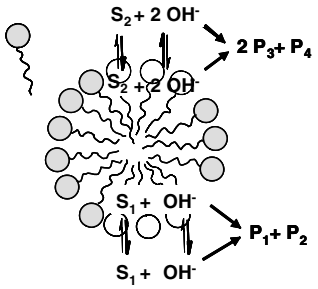


Figure 7. Illustration of how micelles may influence chemical reactions. The micelle is formed by the surfactant sodium decanoate at pH 11.0. Due to a different partitioning between the bulk aqueous medium and the decanoate micelles, the reaction rates for the two substrates S_1 and S_2 are influenced differently. S_1 has a lower affinity for the micelles than S_2 has. The reaction products are P_1 and P_2, and P_3 and P_4, respectively. S_1: p-nitrophenylacetate; S_2: fluorescein diacetate; P_1: acetate; P_2: p-nitrophenolate; P_3: acetate; P_4: fluorescein. Reproduced from (Namani and Walde, 2005) with the permission from the American Chemical Society.

(1) Molecular recognition between the two complementary bases adenine and thymine, the latter as amphiphilic derivative (thyminyloctyl) ammonium bromide can occur in a micellar solution composed of sodium dodecylsulfate (SDS) and acetic acid/water (Nowick and Chen, 1992). The base pairing through hydrogen bonds occurs inside the hydrophobic domains of the micellar aggregates. No hydrogen-bonded base pairing is observed in the absence of the surfactant.
(2) Based on the principles of "micellar inhibition" (Menger and Portnoy, 1967), a simple competitive hydrolysis reaction has been investigated in the presence of decanoate micelles, see Figure 7. The two model substances S_1 and S_2 are both hydrolyzed simultaneously in an alkaline aqueous solution. The rates of hydrolysis depend on the concentration and chemical structure of the two molecules. Since the affinity of S_1 to the micelles is different from the affinity of S_2, the micelles directly influence the relative rate of hydrolysis of the two compounds. The micelles act therefore as a kind of very primitive enzyme model, modifying the rates of two competitive reactions with certain selectivity (Namani and Walde, 2005).
(3) The oxidative radical polymerization of aniline to form polyaniline in acidic aqueous solution – and initiated by ammonium peroxodisulfate – is controlled by the presence of SDBS micelles (Kuramoto and Geniès, 1995). The negatively charged micelles lead to a preorganization of the positively charged aniline and to an acceleration of the reaction due to high local concentrations of aniline on the surface of the micelles.

Instead of micelles, other surfactant aggregates have also been used, and it has been shown that the same principles (solubilization and reaction acceleration/inhibition) apply in these cases too, e. g. solubilization and catalysis (i)

by vesicles (Scrimin, 1996; Chaimovich and Cuccovia, 1997) or (ii) by inverted micelles or oil-in-water microemulsions (Fendler, 1982; Sjöblom *et al*., 1996). Table II lists a number of selected examples where the presence of surfactant assemblies leads to a change in the rates of chemical reactions.

Surfactant aggregates as compartments

Vesicles have been used since many years as simple models for the lipid matrix of biological cells. It may well be that the first cells, or their precursors, resembled a lipid vesicle, enriched with the necessary chemical functionalities (see above). It is worth noting that for the first 3×10^9 years, the size of living organisms was probably mainly below 1 mm (Carroll, 2001), which is actually the size regime of vesicles.

The permeability of vesicle membranes for very large molecules (macromolecules like proteins), for large hydrophilic and non-charged molecules (like glucose) or for small hydrophilic and charged substances (like hydrated Na^+ ions) is generally low. In contrary, the permeability for small neutral molecules (like water, ammonia, urea, methanol) is high. A modulation of the vesicle membrane permeability can be achieved in different ways, for example by varying the vesicle shell composition, e. g. by simply varying the chain length of the lipids (Paula *et al*., 1996), by adding small amounts (at sub-solubilizing concentrations) of a *micelle-forming surfactant* (Treyer *et al*., 2002; Yoshimoto *et al*., 2004), thereby forming more permeable mixed vesicles. If the micelle-forming surfactant is in excess, however, the vesicles will be transformed into (mixed) micelles, accompanied by a complete release of the originally trapped aqueous content.

Another possibility of modifying the vesicle permeability is through a variation of the temperature. Below T_m, the permeability is very low due to the solid-ordered packing of the surfactant molecules. Above T_m, the surfactants are less ordered and the permeability increases. At T_m, the permeability is found to be often highest in the case of metal ions and small molecules (New, 1990; Monnard and Deamer, 2001).

Adding small (or large) membrane soluble molecules, such as ethanol or certain peptides, such as melittin or surfactin (Carrillo *et al*., 2003), and peptide derivatives or RNA (Vlassov *et al*., 2001), may also lead to a change in the permeability barrier properties of the vesicle shell(s). Furthermore, it has for example been shown that the permeability of phospholipid vesicle membranes can be modulated by a hydrocarbon derivative of the simple heptapeptide $(Gly)_3$-L-Pro-$(Gly)_3$, resulting in the formation of chloride selective channels; NO_3^-, SO_4^{2-} and K^+ seem to be transported to a considerably lesser extent than Cl^-(Schlesinger *et al*., 2002).

Another example for specifically modulating the vesicle membrane permeability is the addition of lipophilic antibiotic metal ion carrier calcimycin (also called A23187) which makes the vesicle bilayer permeable for divalent cations like Cu^{2+}, Pb^{2+} and Cd^{2+}, but not so much for Ca^{2+} (Stanish and Monbouquette, 2001).

Due to the semipermeability of lipid vesicles composed of conventional lipids, transient concentration gradients can be established across the vesicle shells. It is

TABLE II

Acceleration or deceleration of reaction rates by micelles, cubic phases, microemulsions and vesicles

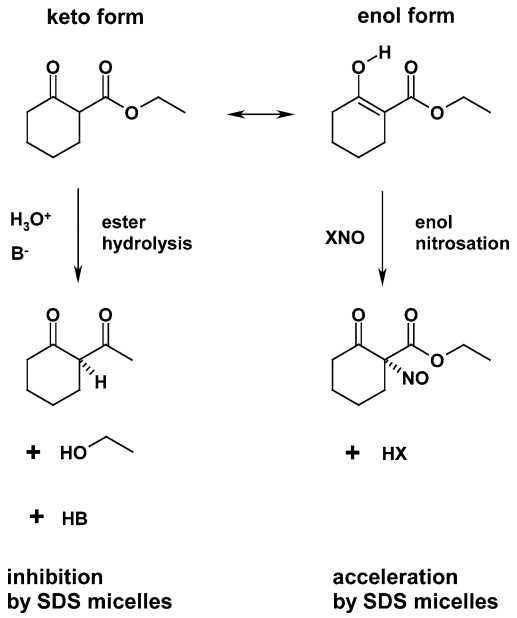
Example 1 (Iglesias, 2001)
Acceleration of the rate of nitrosation of ethyl cyclohexanone-2-carboxylate in the presence of anionic micelles formed from SDS (or hydrogen dodecylsulfate) and deceleration of the acid catalyzed hydrolysis of the same substrate in the presence of the same type of micelles. The presence of micelles can therefore have opposite effects on the same substrate, depending on the reaction investigated, see Scheme 1.
Example 2 (Bunton *et al*., 1993)
Acceleration of the S_N2 reaction of Br^- with methyl naphthalene-2-sulfonate (to yield naphthalene-2-sulfonate and methylbromide) in the presence of cationic CTAB micelles; the acceleration is decreased in the case of mixed micelles containing CTAB and the nonionic surfactant $C_{10}E_4$.
Example 3 (Otto *et al*., 1998; Otto and Engberts, 2000; Rispen and Engberts, 2001)
Acceleration of a Diels-Alder reaction in the presence of micelles formed from zinc didodecylsulfate (combined effects of micelles and the metal ion as Lewis acid) or acceleration in the presence of vesicles formed from a cyclic phosphate ester surfactant with Cu(II) counter ions.
Example 4 (Riepe *et al*., 1999)
Acceleration of a specific self-cleavage reaction in an RNA in the presence of NH_4^+ cations and micelles formed from non-ionic (triton X-100 or ganopol X-100) or zwitterionic surfactants (zwittergent 3–10).
Example 5 (Rispens and Engberts, 2003)
Acceleration of the 1,3-dipolar cycloaddition of benzonitrile and N-benzylmaleimide in the presence of micelles formed from cationic CTAB, see Scheme 2.
Example 6 (Klijn and Engberts, 2003)
Accelerated deprotonation of 5-nitrobenzisoxazole by hydroxide ions in the presence of vesicles formed from cationic surfactants (dioctadecyldimethylammonium chloride); the acceleration is decreased in the case of mixed vesicles containing cationic surfactants and anionic surfactants (sodium didecylphosphate); deceleration is observed in the case of overall negatively charged mixed vesicles.
Example 7 (Blocher *et al*., 1999; Blocher *et al*., 2000)
Polycondensation of amino acids and peptides in the presence of vesicles lead to the formation of longer oligomers than in the absence of vesicles due to a binding of starting materials and products onto the vesicle surface, see Figure 8.
Example 8 (Fanun *et al*., 2001)
Thermal reactions of H-Cys-OH and furfural in the presence of a microemulsion system containing sucrose esters/water/dodecane:R-OH (1:1, mol/mol); the sucrose esters being sucrose stearate and sucrose laurate and R-OH being ethanol or hexanol. The reaction in the presence of microemulsions lead to the formation of products that are not formed in a microemulsion-free system. Similar results have been obtained from similar reaction systems in the presence of bicontinuous cubic phases. The cubic phase acts as a kind of "selective microreactor" (Vauthey *et al*., 2000)

(*Continued on next page*)

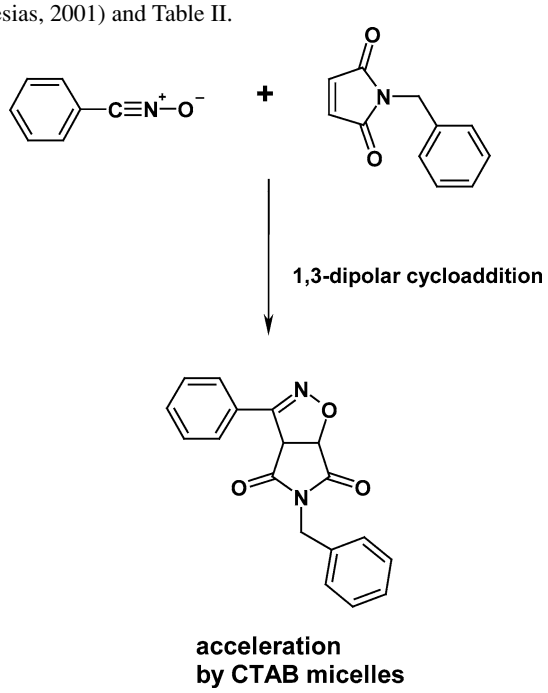
TABLE II
(*Continued*)

Example 9 (Ueoka *et al*., 1988; Ohkubo *et al*., 1995)
Enantioselective hydrolysis of the hydrophobic p-nitrophenylester *n*-dodecanoyl-L-Phe-ONp (or *n*-hexanoyl-L-Phe-ONp) in comparison with the hydrolysis of *n*-dodecanoyl-D-Phe-ONp (or *n*-hexanoyl-D-Phe-ONp) in the presence of oligopeptide catalysts (particularly Z-Phe-His-Leu-OH or Z-Leu-His-Leu-OH) and cationic micelles (particularly the rod like mixed micelles formed from ditetradecyldimethylammonium bromide:hexadecyltrimethylammonium bromide (1:2, mol/mol)). Enantioselectivity is also observed in vesicular systems composed of cationic didodecyldimethylammonium bromide (Ohkubo *et al*., 1995), ditetradecyldimethylammonium bromide (Goto *et al*., 1994) or PC-containing vesicles (Goto *et al*., 1995).
Example 10 (Cleij *et al*., 1991)
Enantioselective hydrolysis of *n*-dodecanoyl-L- (or D-)Phe-containing p-nitrophenyl esters in the presence of hydrophobic L-His-containing dipeptides (e. g. *n*-dodecanoyl-L-Phe-L-His) and the micelle forming chiral, cationic surfactant (*R*) − *N*, *N*-dimethyl-*N*-hexadecyl-*N*-(-methylbenzyl)ammonium bromide).
Example 11 (Ueoka *et al*., 1994)
Diastereoselective hydrolysis of Z-Pro-Pro-ONp and Z-Trp-Pro-ONp in vesicles or mixed micelles (e. g. in vesicles composed of dihexadecyldimethylammonium bromide) and a hydrophobic catalyst (e. g. a iodosobenzoate surfactant).
Example 12 (Scrimin *et al*., 1994)
Enantioselective hydrolysis of amino acid p-nitrophenylesters (particularly the p-nitrophenylester of phenylalanine) in the presence of metallomicelles – micellar aggregates composed of a chiral surfactant and a metal ion (mainly Cu(II)). In some cases, the chiral surfactant is comizellized with CTAB, see Figure 9.
Example 13 (Liu *et al*., 2002)
Enzyme-catalyzed polymerization of aniline in the presence of SDBS micelles and hydrogen peroxide at pH 4.3 leads to the formation of mainly linear polyaniline, while in the absence of SDBS micelles (or other anionic polymeric templates), mainly branched products are formed. Instead of SDBS micelles, mixed SDBS/decanoic acid vesicles can also be used (Namani and Walde, 2005).
Example 14 (Conde-Frieboes and Blöchliger, 2001)
Accelerated transformation of dodecene oxide into a vesicle-forming dialkylphosphate in the presence of phosphate and CTAB micelles.
Example 15 (Cohn *et al*., 2004)
Deceleration of the RNA decomposition by pyrite-induced radicals (e. g. 'OH) if the RNA molecules are encapsulated inside vesicles formed from 1,2-diphytanoyl-*sn*-glycero-3-phosphocholine; coating of pyrite with amphiphiles is efficient as well in slowing down the RNA decomposition.

Selected experimental examples (by no means a complete list), illustrating particular properties of surfactant assemblies in modifying the rates of chemical reactions and the distribution of reaction products. Please note, that the surfactants and reagents used in these examples may not necessarily be of prebiotic relevance, but it is possible that similar processes and principles may have played an important role in prebiotic chemical reactions.



Scheme 1. Effect of SDS micelles on the acid hydrolysis and nitrosation of ethyl cyclohexane-2-carboxylate, see (Iglesias, 2001) and Table II.



Scheme 2. Effect of CTAB micelles on the 1,3-dipolar cycloaddition of benzonitrile oxide and N-benzylmaleimide, see (Rispens and Engberts, 2003), see Table II.

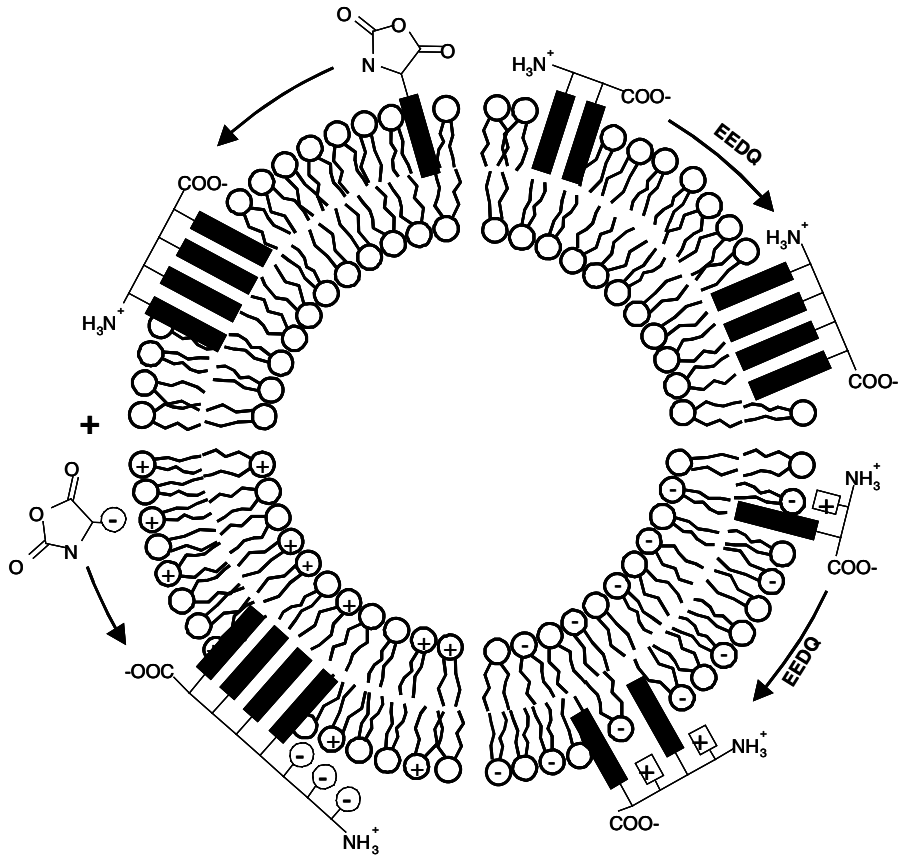


Figure 8. Schematic representation of the vesicle-assisted polycondensation of amino acids and peptides, adopted from (Blocher, 2000). Amino acids with hydrophobic (solid rectangles) and with charged side chains (lower part) were used. The polycondensation was initiated either by using activated amino acids (N-carboxyanhydride amino acids), as illustrated on the left hand side; or by using the hydrophobic condensing agent EEDQ (2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline), as shown on the right hand side. See also (Blocher *et al.*, 1999; Blocher *et al.*, 2000; Hitz *et al.*, 2201), see Table II.

possible that under osmotically balanced conditions, the aqueous solution inside the vesicles is made different form the bulk solution, e. g. inside vesicles formed from egg yolk PC, the initial pH may be 4.0 and outside of the vesicles, the pH may initially be 7.0 (Chakrabarti and Deamer, 1992; Chakrabarti and Deamer, 1994). Such pH gradients across vesicle membranes have been successfully used for example for loading vesicles with amino acid derivatives and peptides or particular drugs for pharmaceutical applications of lipid vesicles as drug delivery vehicle (Chakrabarti *et al.*, 1994; Harrigan *et al.*, 1993). The general principle of this active loading of vesicles by pH gradients is the following (Figure 10). Some of the molecules with

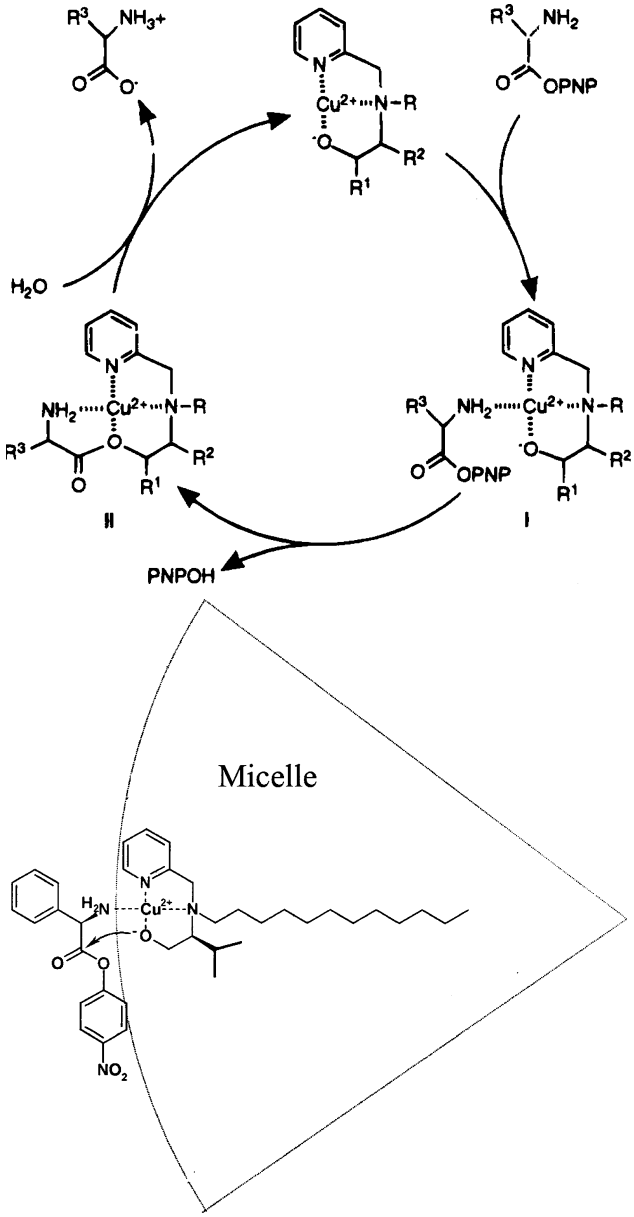


Figure 9. Schematic representation of the mechanism for the enantioselective hydrolysis of amino acid p-nitrophenylester substrates (-OPNP) in the presence of Cu(II)-containing micelles. A: A ternary complex (**I**) is first formed between Cu (II), the surfactant and the substrate, followed by a nucleophilic attack of the (activated) surfactant hydroxyl which leads to a release of p-nitrophenol (PNPOH) and to the formation of the transacylation intermediate (**II**). High enantioselectivity is observed with R: *n*-$C_{16}H_{33}$; R^1: CH_3; R^2: H; R^3: C_6H_5. Adopted from (Scrimin *et al.*, 1994; Cleij *et al.*, 1996). Reproduced from (Scrimin *et al.*, 1994) with permission from the American Chemical Society. B: Visualization of the localization of the substrate within the metallo-micelle. Shown is a part of a spherical micelle, adopted from (Yatsimirsky, 2004), see Table II.

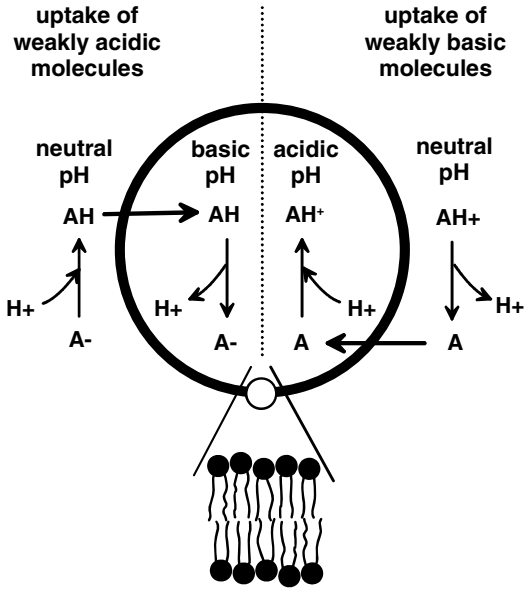


Figure 10. Schematic representation of a cross-section through a spherical unilamellar vesicle composed of *double*-chain surfactants, showing the principles of actively loading the vesicles with externally added weakly basic or weakly acidic molecules. See also (Cullis *et al*., 1997). Explanation for the uptake in the case of weakly basic molecules (right hand side): If the externally present molecule has an appropriate pK_a value, a neutral external pH results in a mixture of both the protonated AH^+ (membrane impermeable) and unprotonated A (membrane permeable) forms of the compound. The neutral form A will tend to diffuse across the membrane until the interior and exterior concentrations are equal. However, the acidic interior pH results in protonation of A (formation of AH^+), thereby driving continued uptake of the compound. Depending on the experimental conditions, a complete uptake of A can be accomplished. The principle of the uptake in the case of weakly acidic molecules (left hand side) is analogous.

which the vesicle compartments are loaded are not charged under the conditions existing outside of the vesicles. Concentration gradients drive the molecules to move at a temperature above T_m from outside to the inside of the vesicles where the molecules get charged due to a different pH. Since the permeability of the charged molecules is much lower in comparison with the uncharged ones, the molecules remain trapped inside the vesicles. A similar principle applies in specific cases by using an ammonium sulfate gradient which induces a pH gradient and eventually leads to conditions under which the loaded compounds (doxorubicin) precipitates inside the vesicles (removed from the chemical equilibrium in solution), see (Haran *et al*., 1993; Barenholz, 2001).

It has been shown that preformed POPC vesicles can be loaded with proteins (enzymes) by simple heat treatment (Umakoshi *et al*., 1998). The increased temperature most likely leads to a conformational change of the protein which in turn

leads to a change in the surface properties of the protein, followed by a translocation across the vesicle membranes.

Preformed sonicated vesicles prepared from DOPC/DOPE (1:1, molar ratio) with diameters below 50 nm (SUV) were used as starting systems to prepare DNA plasmid-loaded vesicles with diameters between 100 and 200 nm. The loading was achieved by using ethanol and Ca^{2+}-ions to fuse the SUV. Entrapment yields of up to 80% were obtained (Bailey and Sullivan, 2000). In a similar work, polynucleotides and oligonucleotides have been entrapped inside vesicles containing cationic lipids by using ethanol. The ethanol concentrations required were very close to the region of breakdown of the vesicle structure (Maurer *et al*., 2001).

More recently, experiments have been carried out that indicated that certain oligopeptides, e. g. penetratin analogs that are known to be taken up by biological cells *in vitro*, are apparently able to translocate across lipid vesicle membranes, depending on the size of the vesicles (Persson *et al*., 2004). Translocation has been observed with giant vesicles of DOPC/DOPG (3:2, molar ratio), but not with LUV.

In summary, all the experiments mentioned demonstrate that preformed vesicular compartments can be loaded with certain substances (small or large) in relatively simple ways by chemical means. It is worth noting that the early work of Stillwell and Winter already dealt with permeability aspects of vesicles as protocell models (Stillwell and Winter, 1973; Stillwell, 1976). In these studies, water-soluble aldehydes were used to increase the membrane permeability for glycine, lysine and histidine (possibly *via* a transient imine formation between the aldehyde and the amino group of the amino acids; the imine being more permeable than the corresponding amino acid). Vesicles may also be loaded with water-soluble molecules during the vesicle formation process (often a simple hydration of dry vesicle-forming surfactants), see (Walde and Ichikawa, 2001; Monnard, 2003). In the case of DNA uptake, the presence of Mg^{2+}-ions may be beneficial to obtain high entrapment yields (Sato *et al*., 2003).

Following earlier experiments with sub-micrometer-sized POPC vesicles (Oberholzer *et al*., 1999), protein expression has been detected in cell-sized vesicle systems: if an appropriate complex reaction mixture – containing all the required substances for gene expression – has been encapsulated within giant vesicles formed from DOPC/DOPG (10:1, molar ratio), expression of the red-shifted green fluorescent protein could be observed within individual vesicles. Apparently, at the early stage of the reaction, the gene expression efficiency inside the vesicles was considerably higher than that in the bulk solution (Nomura *et al*., 2003). In a further extension of this type of work, a cascading genetic network has also been expressed inside giant vesicles (Ishikawa *et al*., 2004). Furthermore, the replication of an RNA template catalyzed by Q_β-replicase inside lipid vesicles has also been achieved (Oberholzer *et al*., 1995).

Table III lists a number of examples in which some of the compartment aspects of vesicles are emphasized (including vesicle fusion, budding and reproduction).

TABLE III

Vesicle formation, fusion, fission, budding and reproduction processes

Example 1 (Hanczyc *et al*., 2003)
Vesicle growth and division processes observed after adding micelles formed from myristoleate (9-*cis*-tetradecenoate) to vesicles composed of myristoleic acid and myristoleate. Clay particles (montmorillonite) accelerate the conversion of micelles to vesicles. For vesicle growth and division in similar fatty acid-based systems, see (Berclaz *et al*., 2001; Chen and Szostak, 2004). For a report on RNA catalysis inside fatty acid-based vesicles, see (Chen *et al*., 2005).
Example 2 (Walde *et al*., 1994; Wick *et al*., 1995)
Hydrolysis of oleic anhydride in the presence of oleic acid/oleate vesicles, resulting in an increase in the number of vesicle aggregates. In rare cases, internal vesicles escape from a giant "mother vesicle" in a process called "vesicle birthing".
Example 3 (Menger and Gabrielson, 1994)
"Vesicle birthing" observed after adding the nonionic surfactant *n*-octylglucoside to a didodecyldimethylammonium bromide giant vesicle that contained a smaller internal vesicle.
Example 4 (Takakura *et al*., 2003)
Giant vesicle compartment formation as a consequence of a condensation reaction occurring in a vesicular system from hydrophilic and hydrophobic precursor structures. The hydrophobic precursor structure initially forms an oil droplet inside a "mother vesicle"; the oil droplet is converted into vesicle-forming amphiphiles that escape from the mother vesicles ("vesicle birthing").
Example 5 (Svetina and Žekš, 2002)
Theoretical understanding of experimental observations about vesicles that can undergo cell-like morphological changes (see Figure 11). It is also found theroretically that the self-reproduction of growing vesicles is based on a selective membrane permeability (Božič and Svetina, 2004).
Example 6 (Menger and Balachander, 1992)
Giant vesicle aggregation, budding, and fusion observed in the case of vesicles formed from didodecyldimethylammonium bromide, after addition of sodium sulfate (aggregation), or sodium acetate (budding and later on fusion).
Example 7 (Döbereiner *et al*., 1993)
Giant vesicle budding observed upon heating vesicles formed from sphingomyelins; osmotically-induced budding and fission observed with DMPC-cholesterol vesicles. The observations are taken as evidence that vesicle fission in biological systems may not necessarily involve proteins.
Example 8 (Käs and Sackmann, 1991)
Different shape transitions observed in giant DMPC or POPC vesicles *via* a variation of the temperature that resulted in changes of the vesicle-surface-area to vesicle-volume ratio; see also (Sackmann, 1994).
Example 9 (Nomura *et al*., 2001)
Different topological transformations of phosphatidylcholine-containing giant vesicles upon addition of different micelle-forming surfactants. The transformations include inside-out inversions and "vesicle-birthing".
Example 10 (Tanaka *et al*., 2004)
Addition of the single-chain phospholipid 1-palmitoyl-2-hydroxy-*sn*-glycero-3-phosphocholine to giant mixed DPPC/cholesterol (6:4, molar ratio) vesicles above a threshold concentration leads to several vesicle shape changes, including fission. The initial physical state of the membrane within the vesicle shells seems to be of importance for the observed vesicle fission.

(*Continued on next page*)

TABLE III
(*Continued*)

Example 11 (Akiyoshi *et al*., 2003)
Formation of tubular structures and vesicle networks upon addition of the amphiphile ganglioside GM3 to giant vesicles formed from DOPC.
Example 12 (Bolinger *et al*., 2004)
Formation of multivesicular vesicles (see Figure 5A) containing within a large vesicle composed of DOPG ($T_m = -18\,^\circ C$) small vesicles prepared from DPPC and DPPG (9:1, molar ratio, $T_m = 41\,^\circ C$). The release of a water-soluble dye (carboxyfluorescein) from the encapsulated vesicles into the aqueous interior of the large vesicle is triggered by increasing the temperature to the chain melting temperature of the surfactants of the encapsulated vesicles (41 °C).

SURFACTANT AGGREGATES AS FOLDING AND REACTIVITY MODIFIERS OF POLYPEPTIDES AND NUCLEIC ACIDS

The interaction of certain polypeptides (proteins) with fluid lipid bilayers (vesicles) or micelles can lead to a change in the conformation of the polypeptides, and as a consequence may induce a certain functionality, for example the formation of specific channels inside a lipid bilayer (Pohorille *et al*., 2003). The lipid membrane can be considered in a first approximation as a kind of special solvent that contributes to the stability and the folding of the particular polypeptides. The interaction of the peptides may be strongly dependent on the structural and elastic properties of the membrane (Hallock *et al*., 2002; McIntosh, 2004).

Surfactant aggregates – reverse micelles (Hagen *et al*., 1990) and vesicles (Kuboi *et al*., 1997; Yoshimoto and Kuboi, 1999) – can function as "primitive chaperons" in the sense that they may catalyze the refolding of denatured proteins. In the case of carbonic anhydrase and vesicles, it is likely that an interaction of the hydrophobic molten globule state of the protein with the vesicles plays an important role for the observed assistance in the refolding process (Kuboi *et al*., 1997), Figure 12.

If the lipid composition of a bilayer vesicle membrane is made slightly asymmetric by adding to the outer monolayer, surfactant molecules that are not components of the vesicle, then the surface tension may be changed (Traïkia *et al*., 2002) which may lead to a change in the biological activity of vesicle bound peptides, e.g. gramicidin (Goulian *et al*., 1998).

A few further examples are given to illustrate how the interactions with surfactant assemblies can lead to a modification of the conformation of polypeptides or nucleic acids: (a) While the folding of T4DNA is controlled by vesicles prepared from cationic amphiphiles (distearoyldimethylammonium bromide), unfolding is observed in the presence of zwitterionic vesicles prepared from soybean PC (Mel'nikov *et al*., 1997); (b) The hairpin conformation of the octapeptide Gly-Ala-Asn-Pro-Asn-Ala-Ala-Gly containing hydrophobic chains at the N- and C-termini is stabilized by vesicles prepared from DSPC (Löwik *et al*., 2003). The secondary structure of N-terminally acylated peptides and their self-assembly

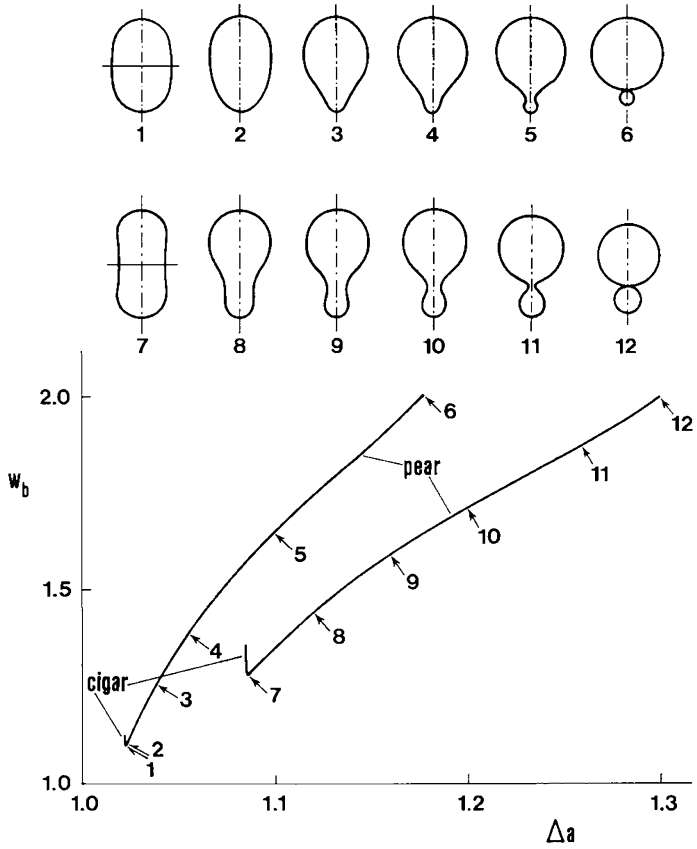


Figure 11. Theoretical understanding and predictions of shape changes of vesicles that are composed of a single bilayer. The bending energy relative to the bending energy of the sphere (w_b) is represented as a function of the relative difference between the areas of the two membrane monolayers (Δa), illustrating how experimentally observed vesicle budding is understood with a simple model, adopted from (Svetina and Žekš, 2002). Vesicle-self-reproduction is described as a process in which a growing vesicle first transforms its shape from a sphere into a budded shape of two spheres connected by a narrow neck, and then splits into two spherical daughter vesicles (Božič and Svetina, 2004), see Table III. Reproduced from (Svetina and Žekš, 2002) with permission from John Wiley & Sons, Inc.

behavior can be tuned by varying the length of the acyl chain in the absence of any vesicles or other surfactant assemblies, illustrating an interplay between self-assembly of amphiphilic peptide derivatives and peptide folding (Löwik *et al.*, 2005); (c) The conformation of the membrane protein BtuB is controlled by the size, composition and curvature of mixed micelles composed of short- and long-chain PCs, dihexanoyl PC and DMPC (Fanucci *et al.*, 2003); (d) The binding of the oligopeptide penetratin to negatively charged vesicles composed of POPC and POPG leads to a change of the peptide conformation. The peptide has a random coil conformation in aqueous solution and adopts a mainly α-helical conformation upon

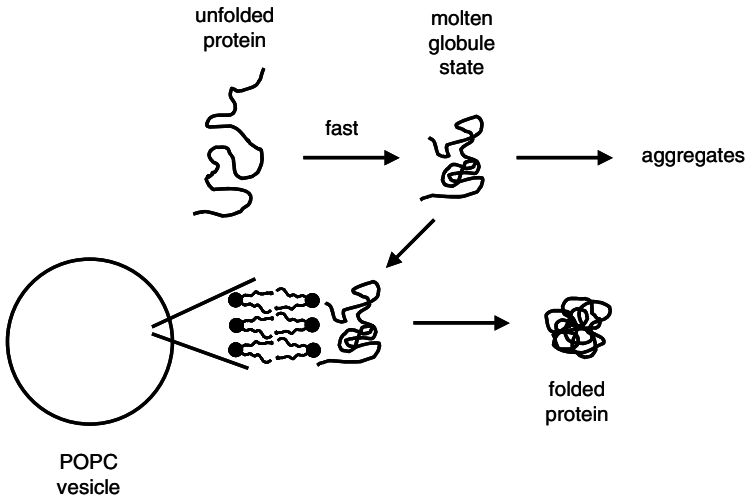


Figure 12. Schematic representation of the vesicle-assisted folding of the enzymatically active protein carbonic anhydrase. In the presence of 5 M guandidinium hydrochloride, the enzyme denatures into its unfolded state. Upon dilution into a suspension containing POPC vesicles, the refolding process is influenced by the vesicles, most likely *via* a binding of the intermediate molten globule state to the outer surface of the vesicles, adopted from (Kuboi *et al*., 1997).

binding to POPC/POPG vesicles at a molar ratio below 3:7 at high lipid to peptide ratios. If the vesicles contain more POPG, the peptide changes its conformation to a β-sheet, illustrating how small changes in the membrane composition may have influence the conformation of adsorbed peptides (Magzoub *et al*., 2002).

Surfactant aggregates as templates

Micellar and hexagonal surfactant aggregates can control the formation of inorganic mesoporous structures. A silicate/aluminiumsilicate mesoporous material has for example been prepared by using cationic surfactants $C_nH_{2n+1}N^+(CH_3)_3$ ($n = 8$, 9, 10, 12, 14, 16) as self-assembled templates (Beck *et al*., 1992). The surfactants used are known to form hexagonal phases at the concentration used. If the surfactants are mixed with inorganic silicates and/or aluminiumsilicates in aqueous solution, followed by an incubation at a temperature of about 150 °C in an autoclave, and an extensive washing of the product obtained at room temperature with water, then a porous inorganic material is obtained with a hexagonal array of rather uniform pores. The pore size depends on the chain length of the surfactant used, varying between ~15 A° to more than 100 A° (Beck *et al*., 1992). Such materials have an enormous surface area like zeolites and may be considered as possible reaction sites for the synthesis of prebiotically relevant molecules (Smith *et al*., 1999).

Vesicles may provide confined reaction compartments for the synthesis of nanocrystals. In the case of CdS, the size of the crystals (2–6 nm) formed from $CdCl_2$ and $(NH_4)_2S$ could be controlled by the amount of $CdCl_2$ entrapped inside the vesicles and the vesicle diameter; the vesicles were prepared from egg yolk PC (Kogel and Monbouquette, 1996). Each vesicle contained one nanocrystal (Kennedy *et al*., 1998). The formation of even more complex nanocrystals inside vesicles is also possible (Korgel and Monbouquette, 2000).

Bicontinuous water-filled microemulsions can be used as preorganized systems for the fabrication of crystalline calcium phosphates with extended reticulated microstructures (Walsh *et al*., 1994). The bicontinuous microemulsions were prepared from mixtures of the cationic surfactant didodecyldimedthylammonium bromide, an aqueous solution, and a long chain alkane (dodecane, tetradecane or hexadecane).

Micelles may act as templates to control the polymerization of monomers in aqueous solution, see Table II (Example 13). In this case, the surfactant aggregates lead to a preorganization of the molecules to be polymerized.

Summary and Concluding Remarks

Questions concerning the origin(s) of life have been asked for centuries and no clear scientific answer has been yet found. What remains are hypotheses which are based on different grounds, and there is an enormous amount of interesting literature dealing with different aspects related to the question of the origin(s) of life. Due to the interdisciplinary nature of the general problem of understanding life's origin(s), experimental results and ideas have been collected from a number of different disciplines (including biophysics, geochemistry, organic and inorganic chemistry, molecular biology, astrobiology, etc.). Only those efforts which are aimed at merging different ideas, different concepts and different ways of thinking will probably lead to a better understanding of living systems and of the mechanisms which led to the formation of the first cells. However, we may never know how these cells looked or under which environmental conditions they first formed. The RNA world hypothesis is attractive, even if there are many open questions with respect to the prebiotic chemical synthesis and stability of oligo-and polynucleotides.

Looking critically to the present state of knowledge in the field and looking back to the work of Oparin (Oparin, 1938; Oparin and Gladilin, 1980), one is tempted to say that since Oparin's ideas progress has been made in the development of certain models (e. g. RNA world, iron-sulfur world, vesicles as protocells models, "lipid-world, etc.). This progress is, however, only a relatively minor. Important aspects of living systems are still not understood, and the development of new concepts will probably be needed.

The present paper is just a modest contribution toward a different way of thinking that has emerged during the last few years (Szostak *et al*., 2001; Segré *et al*., 2001; Luisi *et al*., 2002). Surfactant aggregates have a great potential for providing

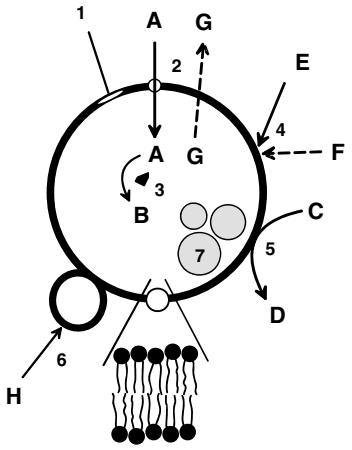


Figure 13. Schematic representation of different properties of surfactant assemblies as illustrated in the case of a hypothetical functionalized bilayered unilamellar vesicle. **1:** Membrane composed of a mixture of bilayer-forming amphiphiles, allowing the formation of domains within the membrane with 'solid-ordered' and 'liquid-disordered' characteristics; **2:** Selective membrane permeability, permeable for the substance A, less permeable for G, achieved by the coaddition of membrane-soluble compounds; **3:** Entrapped catalyst or template (polypeptides, proteins, nucleic acids or inorganic nanoparticles), catalyzing the transformation of A into B; **4:** Selective adsorption of externally present molecules, controlled by the fluidity, hydrophobicity, charge or curvature of the membrane. The substance E adsorbs more efficiently than F; **5:** Enhancement of the reaction rate for the chemical transformation of the substance C into D ("vesicle catalysis"); **6:** Chemical processes resulting in vesicle size and morphology changes and possibly in vesicle budding and fission; **7:** Internal compartments (oil droplets or vesicles) as reservoirs for the triggered release of their contents into the vesicles interior.

sub-micrometer-sized environments for chemical reactions, starting material and product adsorption, for catalysis and for compartmentalization. In contrast to minerals, surfactant aggregates can provide solid as well as liquid surfaces which are charged or uncharged, and range from rigid to elastic in nature. The different aspects of surfactant aggregates should be taken into account in experimental and theoretical studies on prebiological processes. Figure 13 summarizes schematically some of the different aspects of surfactant assemblies as discussed in this article in the case of a bilayered vesicle. Generally, one has to be open for new ideas that may initially seem strange, e.g., the idea of the possible existence of "proto-RNA molecules" (Hud and Anet, 2000) or the hypothesis that mineral membranes made from iron sulfide may have played an important role as compartment structures (Martin and Russell, 2002), in addition to the more conventional vesicles from surfactants described in this article. There remains much to be learnt from the self-organization of mixtures of amphiphilic molecules and from amphiphilic molecules that have chemically complex hydrophilic parts (e.g., chiral surfactants). Some of the chiral assemblies described (Figure 6) represent interesting non-covalent aggregates that

resemble DNA. Most what is discussed in this article is strongly related to the "lipid world" scenario (Segré *et al*., 2001). The idea of an early "lipid world" that preceded an "RNA world" is attractive and the enormous potential of surfactants for controlling various chemical processes is outlined in the present paper to further promote serious consideration s of amphiphilic molecules as early "key players" in the origin(s) of life.

In addition, a new look into the origin and evolution of viruses and the so-called viral form of life – as recently proposed (Bamford, 2003) – may also help in better understanding the origin(s) of life. As pointed out in the cited work, some of the known viruses are in principle nothing else than functionalized vesicles. Some of the virus mimicking particles prepared and investigated, called virosomes, are indeed based on lipid vesicles (Zurbriggen, 2003).

Contemporary life is very complex. The spontaneous transformation of non-living matter to living entities is considered to be a result of a continuous prebiological process over millions of years during which the molecular and supramolecular complexity increased. It may be that the field of supramolecular chemistry, to which the self-association of amphiphilic molecules belongs, may contribute to some extent. Furthermore, it is expected that developments and applications of theories on complexity and research in the fields of systems biology and systems chemistry (von Kiedrowski, 2005) will further contribute to the scientific understanding of life and its origin(s).

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References

Akiyoshi, K., Itaya, A., Nomura, S.-I. M., Ono, N. and Yoshikawa, K.: 2003, Induction of Neuron-like Tubes and Liposome Networks by Cooperative Effect of Gangliosides and Phospholipids, *FEBS Lett*. **534**, 33–38.

Anet, F. A. L.: 2004, The Place of Metabolism in the Origin of Life, *Curr. Opin. Chem. Biol*.**8**, 654–659.

Apel, C. E. and Deamer, D. W.: 2005, The Formation of Glycerol Monodecanoate by a Dehydration/Condensation Reaction: Increasing the Chemical Complexity of Amphiphiles on the Early Earth,*Origins Life Evol. Biospheres* **35**, 323–332.

Ariga, K., Yuki, H., Kikuchi, J., Dannemuller, O., Albrecht-Gary, A.-M., Nakatani, Y. and Ourisson, H.: 2005, Monolayer Studies of Single-Chain Polyprenylphosphates, *Langmuir* **21**, 4578–4583.

Bachmann, P. A., Luisi, P. L. and Lang, J.: 1992, Autocatalytic Self-Replicating Micelles as Models for Prebiotic Structures, *Nature* **357**, 57–59.

Bailey, A. L. and Sullivan, S. M.: 2000, Efficient Encapsulation of DNA Plasmids in Small Neutral Liposomes Induced by Ethanol and Calcium, *Biochim. Biophys. Acta* **1468**, 239–252.

Bamford, D. H.: 2003, Do Viruses form Lineages Across Different Domains of Life?, *Res. Microbiol.* **154**, 231–236.

Barenholz, Y.: 2001, Liposome Application: Problems and Prospects, *Curr. Opin. Colloid Interf. Sci.* **6**, 66–77.

Beck, J. S., Vartuli, J. C., Roth, W. J., Leonowicz, M. E., Kresge, C. T., Schmitt, K. D., Chu, C. T.-W., Olson, D. H., Sheppard, E. W., McCullen, S. B., Higgins, J. B. and Schlenker, J. L.: 1992, A New Family of Mesoporous Molecular Sieves Prepared with Liquid Crystal Templates, *J. Am. Chem. Soc.* **114**, 10834–10843.

Berclaz, N., Müller, M., Walde, P. and Luisi, P. L.: 2001, Growth and Transformation of Vesicles Studied by Ferritin Labeling and Cryotransmission Electron Microscopy, *Langmuir* **105**, 1056–1064.

Bertoncin, F., Mancin, F., Scrimin, P., Tecilla, P. and Tonellato, U.: 1998, Kinetic Amplification of the Enantioselective Cleavage of α-Amino Acid Esters by Metallomicelles, *Langmuir* **14**, 975–978.

Blocher, M. C.: 2000, *Liposome-Assisted Polycondensation of Amino Acids and Peptides*, Dissertation ETH-Zürich, No. 13591.

Blocher, M., Liu, D., Walde, P. and Luisi, P. L.: 1999, Liposome-Assisted Selective Polycondensation of Amino Acids and Peptides, *Macromolecules* **32**, 7332–7334.

Blocher, M., Liu, D. and Luisi, P. L.: 2000, Liposome Assisted Selective Polycondensation of α-Amino Acids and Peptides: The Case of Charged Liposomes, *Macromolecules* **33**, 5787–5796.

Bolinger, P.-Y., Stamou, D. and Vogel, H.: 2004, Integrated Nanoreactor Systems: Triggering the Release and Mixing of Compounds Inside Single Vesicles, *J. Am. Chem. Soc.* **126**, 8594–8595.

Bolli, M., Micura, R. and Eschenmoser, A.: 1997, Pyranosyl-RNA: Chiroselective Self-Assembly of Base Sequences by Ligative Oligomerization of Tetranucleotide-2′,3′-Cyclophosphates (with a Commentary Concerning the Origin of Biomolecular Homochirality), *Chem. & Biol.* **4**, 309–320.

Bonaccio, S., Walde, P. and Luisi, P. L.: 1994, Liposomes Containing Purine and Pyrimidine Bases: Stable Unilamellar Liposomes from Phosphatidyl-Nucleosides, *J. Phys. Chem.* **98**, 6661–6663 and 10376.

Bonaccio, S., Wessiken, M., Berti, D., Walde, P. and Luisi, P. L.: 1996, Relation Between the Molecular Structure of Phosphatidyl Nucleosides and the Morphology of their Supramolecular and Mesoscopic Aggregates, *Langmuir* **12**, 4976–4978.

Bonner, W. A.: 1999, Chirality Amplification – the Accumulation Principle Revisited, *Origins Life Evol. Biosphere* **29**, 615–623.

Božič, B. and Svetina, S.: 2004, A Relationship Between Membrane Properties Forms the Basis of a Selectivity Mechansim for Vesicle Self-Reproduction, *Eur. Biophys. J.* **33**, 565–571.

Buijnsters, P. J. J. A., Feiters, M. C., Nolte, R. J. M., Sommerdijk, N. A. J. M. and Zwanenburg, B.: 2001, Autocatalytic Ring Opening of N-Acylaziridines. Complete Control Over Regioselectivity by Orientation at Interfaces, *Chem. Commun.* 269–270.

Bunton, C. A., Nome, F., Quina, F. H. and Romsted, L. S.: 1991, Ion Binding and Reactivity at Charged Aqueous Interfaces, *Acc. Chem. Res.* **24**, 357–364.

Bunton, C. A., Wright, S., Holland, P. M. and Nome, F.: 1993, S_N2 Reactions of a Sulfonate Ester in Mixed Cationic/Nonionic Micelles, *Langmuir* **9**, 117–120.

Cairns-Smith, A. G.:1985, *Seven Clues to the Origin of Life*, Cambridge University Press, Cambridge, UK.

Carrillo, C., Teruel, J. A., Aranda, F. J. and Ortiz, A.: 2003, Molecular Mechanism of Membrane Permeabilization by the Peptide Antibiotic Surfactin, *Biochim. Biophys. Acta* **1611**, 91–97.

Carroll, S. B.: 2001, Chance and Necessity: The Evolution of Morphological Complexity and Diversity, *Nature* **409**, 1102–1109.

Cescato, C., Walde, P. and Luisi, P. L.: 1997, Supramolecular Transformations of Vesicles from Amino Acid Based Double Chain Amphiphiles, *Langmuir* **13**, 4480–4482.

Chaimovich, H. and Cuccovia, I. M.: 1997, Quantitative Analysis of Reagent Distribution and Reaction Rates in Vesicles, *Progr. Colloid Polym. Sci.* **103**, 67–77.

Chakrabarti, A. C., Clark-Lewis, I. and Cullis, P. R.: 1994, Influence of Charge, Charge Distribution, and Hydrophobicity on the Transport of Short Model Peptides into Liposomes in Response to Transmembrane pH Gradients, *Biochemistry* **33**, 8479–8485.

Chakrabarti, A. C. and Deamer, D. W.: 1992, Permeability of Lipid Bilayers to Amino Acids and Phosphates, *Biochim. Biophys. Acta* **1111**, 171–177.

Chakrabarti, A. C. and Deamer, D. W.: 1994, Permeation of Membranes by the Neutral form of Amino Acids and Peptides: Relevance to the Origin of Peptide Translocation, *J. Mol. Evol.* **39**, 1–5.

Chen, I. A. and Szostak, J. W.: 2004, A Kinetic Study of the Growth of Fatty Acid Vesicles, *Biophys. J.* **87**, 988–998.

Chen, I. A., Salehi-Ashtiani, K. and Szostak, J. W.: 2005, RNA Catalysis in Model Protocell Vesicles, *J. Am. Chem. Soc.***127**, 13213–13219.

Cleij, M. C., Drenth, W. and Nolte, R. J. M.: 1991, Mechanism of Enantioselective Ester Cleavage by Histidine-Containing Dipeptides at a Micellar Interface, *J. Org. Chem.* **56**, 3883–3891.

Cleij, M. C., Scrimin, P., Tecilla, P. and Tonellato, U.: 1996, Chiral Lipophilic Ligands. 3. Control of Enantioselectivity in Copper(II)-Catalyzed Cleavage of Amino Acid Esters by Aggregate Morphology, *Langmuir* **12**, 2956–2960.

Cohn, C. A., Borda, M. J. and Schoonen, M. A.: 2004, RNA Decomposition by Pyrite-Induced Radicals and Possible Role of Lipids During the Emergence of Life, *Earth Planet. Sci. Lett.* **225**, 271–278.

Conde-Frieboes, K. and Blöchliger, E.: 2001, Synthesis of Lipids on the Micelle/Water Interface Using Inorganic Phosphate and an Alkene Oxide, *BiSystems* **61**, 109–114.

Corliss, J. B., Baross, J. A. and Hoffman, S. E.: 1981, An Hypothesis Concerning the Relationship Between Submarine Hot Springs and the Origin of Life on Earth, *Ocean. Acta* **4**, Suppl. 59–69.

Cullis, P. R., Hope, M. J., Bally, M. B., Madden, T. D., Mayer, L. D. and Fenske, D. B.: 1997, Influence of pH Gradients on the Transbilayer Transport of Drugs, Lipids, Peptides and Metal Ions Into Large Unilamellar Vesicles, *Biochim. Biophys. Acta* **1331**, 187–211.

Deamer, D. W.: 1985, Boundary Structures are Formed by Organic Components of the Murchison Carbonaceous Chondrite, *Nature* **317**, 792–794.

Deamer, D. W.: 1997, The First Living Systems: A Bioenergetic Perspective, *Microbiol. Mol. Biol. Rev.* **61**, 239–261.

Deamer, D., Dworkin, J. P., Sandford, S. A., Bernstein, M. P. and Allamandola, L. J.: 2002, The First Cell Membranes, *Astrobiology* **2**, 371–381.

de Duve, C.: 1995, *Vital Dust: Life as a Cosmic Imperative,* Basic Books, New York.

Döbereiner, H.-G., Käs, J., Noppl, D., Sprenger, I. and Sackmann, E.: 1993, Budding and Fission of Vesicles, *Biophys. J.* **65**, 1396–1403.

Doolittle, W. F. and Brown, J. R.: 1994, Tempo, Mode, the Progenote, and the Universal Root, *Proc. Natl. Acad. Sci. USA* **91**, 6721–6728.

Doudna, J. A. and Cech, T. R.: 2002, The Chemical Repertoire of Natural Ribozymes, *Nature* **418**, 222–228.

Dubois, M., Demé, B., Gulik-Krzywicki, T., Dedieu, J.-C., Vautrin, C., Désert, S., Perez, E. and Zemb, T.: 2001, Self-Assembly of Regular Hollow Icosahedra in Salt-Free Catanionic Solutions, *Nature* **411**, 672–675.

Dubois, M., Lizunov, V., Meister, A., Gulik-Krzywicki, T., Verbavatz, J. M., Perez, E., Zimmerberg, J. and Zemb, T.: 2004, Shape Control Through Molecular Segregation in Giant Surfactant Aggregates, *Proc. Natl. Acad. Sci. USA* **101**, 15082–15087.

Dyson, F.: 1999, *Origins of Life*, 2nd ed., Cambridge University Press, New York.
Edwards, M. R.: 1998, From a Soup or a Seed? Pyritic Metabolic Complexes in the Origin of Life, *Trends Ecol. Evol.* **13**, 178–181.
Epps, D. E., Sherwood, E., Eichberg, J. and Oró, J.: 1978, Cyanamide Mediated Syntheses Under Plausible Primitive Earth Conditions. V. The Synthesis of Phosphatidic Acids, *J. Mol. Evol.* **11**, 279–292.
Fanucci, G. E., Lee, J. Y. and Cafiso, D. S.: 2003, Membrane Mimetic Environments Alter the Conformation of the Outer Membrane Protein BtuB, *J. Am. Chem. Soc.* **125**, 13932–13933.
Fanun, M., Leser, M., Aserin, A. and Garti, N.: 2001, Sucrose Ester Microemulsions as Microreactors for Model Maillard Reaction, *Colloids Surf. A* **194**, 175–187.
Fendler, J. H.: 1982, *Membrane Mimetic Chemistry: Characterizations and Applications of Micelles, Microemulsions, Monolayers, Bilayers, Vesicles, Host-Guest Systems, and Polyions*, John Wiley & Sons, New York.
Fletcher, P. D. I. and Robinson, B. H.: 1984, Effect of Organised Surfactant Systems on the Kinetics of Metal-Ligand Complex Formation and Dissociation, *J. Chem. Soc. Faraday Trans. 1* **80**, 2417–2437.
Flynn, G. J., Keller, L. P., Jacobsen, C. and Wirick, S.: 2004, An Assessment of the Amount and Types of Organic Matter Contributed to the Earth by Interplanetary Dust, *Adv. Space Res.* **33**, 57–66.
Fry, I.: 2000, *The Emergence of Life on Earth: A Historical and Scientific Overview*, Rutgers University Press, New Brunswick.
Fuhrhop, J.-H. and Helfrich, W.: 1993, Fluid and Solid Fibers Made of Lipid Molecular Bilayers, *Chem. Rev.* **93**, 1565–1582.
Furnes, H., Banerjee, N. R., Muehlenbachs, K., Staudigel, H. and de Wit, M.: 2004, Early Life Recorded in Archean Pillow Lavas, *Science* **304**, 578–581.
Gánti, T.: 2003*, The Principles of Life*, with a Commentary by J. Griesemer, and E. Szathmáry, Oxford University Press.
Gilbert, W.: 1986, The RNA World, *Nature* **319**, 618.
Goto, K., Imamura, C., Yamamoto, S., Matsumoto, Y. and Ueoka, R.: 1994, Remarkable Salt Effects in the Highly Enhanced Enantioselective Hydrolysis of Amino Acid Esters with the Active Tripeptide in the Vesicular System, *Chem. Lett.* 2081–2084
Goto, K., Matsumoto, Y. and Ueoka, R.: 1995, Hybrid Liposomes Coupled to Steric Control with High Enantioselectivity, *J. Org. Chem.* **60**, 3342–3346.
Goulian, M., Mesquita, O. N., Fygenseon, D. K., Nelsen, C., Andersen, O. S. and Libchaber, A.: 1998, Gramicidin Channel Kinetics Under Tension, *Biophys. J.* **74**, 328–337.
Hagen, A. J., Hatton, T. A. and Wang, D. I. C.: 1990, Protein Refolding in Reversed Micelles, *Biotechnol. Bioeng.* **35**, 955–965.
Hallock, K. J., Lee, D.-K., Omnaas, J., Mosberg, H. I. and Ramamoorthy, A.: 2002, Membrane Composition Determines Paradaxin's Mechanism of Lipid Bilayer Disruption, *Biophys. J.* **83**, 1004–1013.
Hanczyc, M. M., Fujikawa, S. M. and Szostak, J. W.: 2003, Experimental Models of Primitive Cellular Compartments: Encapsulation, Growth, and Division, *Science* **302**, 618–622.
Haran, G., Cohen, R., Bar, L. K. and Barenholz, Y.: 1993, Transmembrane Ammonium Sulfate Gradients in Liposomes Produce Efficient and Stable Entrapment of Amphipathic Weak Bases, *Biochim. Biophys. Acta* **1151**, 201–215.
Hargreaves, W. R., Muulvihill, S. J. and Deamer, D. W.: 1977, Synthesis of Phospholipids and Membranes in Prebiotic Conditions, *Nature* **266**, 78–80.
Harrigan, P. R., Wong, K. F., Redelmeier, T. E., Wheeler, J. J. and Cullis, P. R.: 1993, Accumulation of Doxorubicin and Other Lipophilic Amines into Large Unilamellar Vesicles in Response to Transmembrane pH Gradients, *Biochim. Biophys. Acta* **1149**, 329–338.

Hiscox, J. A.: 2001, An Overview on the Origin of Life: The case for Biological Prospecting on Mars, *Earth, Moon Planets* **87**, 191–212.

Hitz, T., Blocher, M., Walde, P. and Luisi, P. L.: 2001, Stereoselectivity Aspects in the Condensation of Racemic NCA-Amino Acids in the Presence and Absence of Liposomes, *Macromolecules* **34**, 2443–2449.

Hud, N. V. and Anet, F. A. L.: 2000, Intercalation-Mediated Synthesis and Replication: A New Approach to the Origin of Life, *J. Theor. Biol.* **205**, 543–562.

Iglesias, E.: 2001, Ethyl Cyclohexanone-2-carboxylate in Aqueous Micellar Solutions. 2. Enol Nitrosation in Anionic and Cationic Micelles, *J. Phys. Chem. B* **105**, 10295–10302.

Imae, T., Hayashi, N., Matsumoto, T., Tada, T. and Furusaka, M.: 2000, Structures of Fibrous Supramolecular Assemblies Constructed by Amino Acid Surfactants: Investigation by AFM, SANS, and SAXS, *J. Colloid Interf. Sci.* **225**, 285–290.

Ishikawa, K., Sato, K., Shima, Y., Urabe, I. and Yomo, T.: 2004, Expression of a Cascading Genetic Network Within Liposomes, *FEBS Lett.* **576**, 387–390.

Itojima, Y., Ogawa, Y., Tsuno, K., Handa, N. and Yanagawa, H.: 1992, Spontaneous Formation of Helical Strands from Phospholipid-Nucleoside Conjugates, *Biochemistry,* **31**,4757–4765.

Joyce, G. F., Schwartz, A. A., Miller, S. L. and Orgel, L. E.: 1987, The Case of an Ancestral Genetic System Involving Simple Analogues of the Nucleotides, *Proc. Natl. Acad. Sci. USA* **84**, 4398–4402.

Jung, H. T., Coldren, B., Zasadzinski, J. A., Iampietro, D. J. and Kaler, E. W.: 2001, The Origins of Stability of Spontaneous Vesicles, *Proc. Natl. Acad. Sci. USA* **98**, 1353–1357.

Kaler, E. W., Murthy, A. K., Rodriguez, B. E. and Zasadzinski, J. A. N.: 1989, Spontaneous Vesicle Formation in Aqueous Mixtures of Single-Tailed Surfactants, *Science* **245**, 1371–1374.

Kaler, E. W., Herrington, K. L., Murthy, A. K. and Zasadzinski, J. A. N.: 1992, Phase Behavior and Structures of Mixtures of Anionic and Cationic Surfactants, *J. Phys. Chem.* **96**, 6698–6707.

Käs, J. and Sackmann, E.: 1991, Shape Transitions and Shape Stability of Giant Phospholipid Vesicles in Pure Water Induced by Area-to-Volume Changes, *Biophys. J.* **60**, 825–844.

Kennedy, M. T., Korgel, B. A., Monbouquette, H. G. and Zasadzinski, J. A.: 1998, Cryo-Transmission Electron Microscopy Confirms Controlled Synthesis of Cadmium Sulfide Nanocrystals within Lecithin Vesicles, *Chem. Mater.* **10**, 2116–2119.

Klijn, J. E. and Engberts, J. B. F. N.: 2003, Kemp Elimination in Membrane Mimetic Reaction Media: Probing Catalytic Properties of Catanionic Vesicles Formed from Double-Tailed Amphiphiles, *J. Am. Chem. Soc.* **125**, 1825–1833.

Köning, J., Boettcher, C., Winkler, H., Zeitler, E., Talmon, Y. and Fuhrhop, J.-H.: 1993, Magic Angle (54.7°) Gradient and Minimal Surfaces in Quadruple Micellar Helices, *J. Am. Chem. Soc.* **113**, 693–700.

Korgel, B. A. and Monbouquette, H. G.: 2000, Controlled Synthesis of Mixed Core and Layered (Zn,Cd)S and (HG,Cd)S Nanocrystals within Phosphatidylcholine Vesicles, *Langmuir* **16**, 3588–3594.

Kuboi, R., Yoshimoto, M., Walde, P. and Luisi, P. L.: 1997, Refolding of Carbonic Anhydrase by 1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine Liposomes, *Biotechnol. Prog.* **13**, 828–836.

Kunitake, T.: 1992, Synthetic Bilayer-Membranes: Molecular Design, Self-Organization, and Application, *Angew. Chem. Int. Ed.* **31**, 709–726.

Kuramoto, N. and Geniès, E. M.: 1995, Micellar Chemical Polymerization of Aniline, *Synth. Met.* **68**, 191–194.

Lahav, N.: 1999, *Biogenesis: Theories of Life's Origins*, Oxford University Press, New York.

Larsson, K.: 1989, Cubic Lipid-Water Phases: Structures and Biomembrane Aspects, *J. Phys. Chem.* **93**, 7304–7314.

Lasic, D. D., Joannic, R., Keller, B. C., Frederik, P. M. and Auvray, L.: 2001, Spontaneous Vesiculation, *Adv. Colloid Interf. Sci.* **89–90**, 337–349.

Lequeux, F. and Candau, S. J.: 1994, Dynamical Properties of Wormlike Micelles, in C. A. Herb, R. K. Prud'homme, (eds), *Structure and Flow in Surfactant Solutions*, ACS Symposium Series 578, American Chemical Society, Washington, DC, pp. 51–62.

Lindblom, G. and Rilfors, L.: 1989, Cubic Phases and Isotropic Structures Formed by Membrane Lipids-Possible Biological Relevance, *Biochim. Biophys. Acta* **988**, 221–256.

Liu, W., Kumar, J., Tripathy, S. and Samuelson, L. A.: 2002, Enzymatic Synthesis of Conducting Polyaniline in Micelle Solutions, *Langmuir* **18**, 9696–9704.

Löwik, D. W. P. M., Garcia-Hartjes, J., Meijer, J. T. and van Hest, J. C. M.: 2005, Tuning Secondary Structure and Self-Assembly of Amphiphilic Peptides, *Langmuir* **21**, 524–526.

Löwik, D. W. P. M., Linhardt, J. G., Adams, P. J. H. M. and van Hest, J. C. M.: 2003, Non-Covalent Stabilization of a Hairpin Peptide into Liposomes, *Org. Biomol. Chem.* **1**, 1827–1829.

Luisi, P. L.: 2000, The Relevance of Supramolecular Chemistry for the Origin of Life, *Adv. Supramol. Chem.* **6**, 287–307.

Luisi, P. L.: 2002a, Toward the Engineering of Minimal Living Cells, *Anat. Rec.* **268**, 208–214.

Luisi, P. L.: 2002b, Emergence in Chemistry: Chemistry as the Embodiment of Emergence, *Foundations of Chemistry* **4**, 183–200.

Luisi, P. L. and Walde, P., (eds): 2000, *Giant Vesicles*, Perspectives in Supramolecular Chemistry, Vol. 6, Wiley, Chichester.

Luisi, P. L., Oberholzer, T. and Lazcano, A.: 2002, The Notion of a DNA Minimall Cell: A General Discourse and Some Guidelines for an Experimental Approach, *Helv. Chim. Acta* **85**, 1759–1777.

Magzoub, M., Eriksson, L. E. G. and Gräsland, A.: 2002, Conformational States of the Cell-Penetrating Peptide Penetratin When Interacting with Phospholipid Vesicles: Effects of Surface Charge and Peptide Concentration, *Biochim. Biophys. Acta* **1563**, 53–63.

Margulis, L.: 1993, *Symbiosis in Cell Evolution: Microbial Communities in the Archean and Proterozoic Eons*, 2nd (ed), W. H. Freeman and Company, New York.

Martin, W. and Russell, M. J.: 2002, On the Origins of Cells: A Hypothesis for the Evolutionary Transitions from Abiotic Geochemistry to Chemoautotrophic Prokaryotes, and from Prokaryotes to Nucleated Cells, *Phil. Trans. R. Soc. Lond. B* **358**, 59–85.

Maurer, N., Wong, K. F., Stark, H., Louie, L., McIntosh, D., Wong, T., Scherrer, P., Semple, S. C. and Cullis, P. C.: 2001, Spontaneous Entrapment of Polynucleotides upon Electrostatic Interaction with Ethanol-Destabilized Cationic Liposomes, *Biophys. J.* **80**, 2310–2326.

Maynard Smith, J. and Szathmáry, E.: 1999, *The Origins of Life: Life from the Birth of Life to the Origins of Language*, Oxford University Press, Oxford.

McCollom, T. M., Ritter, G. and Simoneit, B. R. T.: 1999, Lipid Synthesis Under Hydrothermal Conditions by Fischer-Tropsch-Type Reactions, *Origins Life Evol. Biosphere* **29**, 153–166.

McIntosh, T. J.: 2004, The 2004 Biophysical Society-Avanti Award in Lipids Address: Roles of Bilayer Structure and Elastic Properties in Peptide Localization in Membranes, *Chem. Phys. Lipids* **130**, 83–98.

Mel'nikov, S. M., Sergeyev, V. G., Mel'nikova, Y. S. and Yoshikawa, K.: 1997, Folding of Long DNA Chains in the Presence of Distearyldimethylammonium Bromide and Unfolding Induced by Neutral Liposomes, *J. Chem. Soc., Faraday Trans.* **93**, 283–288.

Menger, F. M: 1991, Groups of Organic Molecules that Operate Collectively, *Angew. Chem. Int. Ed.* **30**, 1086–1099.

Menger, F. M. and Balachander, N.: 1992, Chemically-Induced Aggregation, Budding, and Fusion in Giant Vesicles: Direct Observation by Light Microscopy, *J. Am. Chem. Soc.* **114**, 5862–5863.

Menger, F. M. and Gabrielson, K.: 1994, Chemically-Induced Birthing and Foraging in Vesicle Systems, *J. Am. Chem. Soc.* **116**, 1567–1568.

Menger, F. M. and Portnoy, C. E.: 1967, On the Chemistry of Reactions Proceeding inside Molecular Aggregates, *J. Am. Chem. Soc.* **89**, 4698–4703.

Miller, S. L.: 1998, The endogenous synthesis of organic compounds, in A. Brack, (ed.), *The Molecular Origins of Life: Assembling Pieces of the Puzzle*, Cambridge University Press, Cambdrige, UK, pp. 59-85.

Monnard, P.-A.: 2003, Liposome-Entrapped Polymerases as Models for Microscale/Nanoscale Bioreactors, *J. Membr. Biol.* **191**, 87–97.

Monnard, P.-A. and Deamer, D. W.: 2001, Nutrient Uptake by Protocells: A Liposome Model System, *Origins Life Evol. Biosphere* **31**, 147–155.

Moore, P. B. and Steitz, T. A.: 2002, The Involvement of RNA in Ribosome Function, *Nature* **418**, 229–235.

Moreau, L., Barthélemy, P., El Maataoui, M. and Grinstaff, M. W.: 2004, Supramolecular Assemblies of Nucleoside Phosphocholine Amphiphiles, *J. Am. Chem. Soc.* **126**, 7533–7539.

Morigaki, K., Dallavalle, S., Walde, P., Colonna, S. and Luisi, P. L.: 1997, Autopoietic Self-Reproduction of Chiral Fatty Acid Vesicles, *J. Am. Chem. Soc.* **119**, 292–301.

Morowitz, H. J.: 1992, *Beginnings of Cellular Life: Metabolism Recapitulates Biogenesis*, Yale University Press, New Haven.

Morowitz, H. J., Heinz, B. and Deamer, D. W.: 1988, The Chemical Logic of a Minimum Protocell, *Origins Life Evol. Biosphere* **18**, 281–287.

Mushegian, A.: 1999, The Minimal Genome Concept, *Curr. Opin. Gen. Develop.* **9**, 709–714.

Nakashima, N., Ando, R., Muramatsu, T. and Kunitake, T.: 1994, Unusually Large Induced Circular Dichroism of an Aromatic Compound Bound to Helical Superstructures of Chiral Ammonium Bilayers, *Langmuir* **10**, 232–234.

Nakanishi, N., Asakuma, S. and Kunitake, T.: 1985, Optical Microscopy Study of Helical Superstructures of Chiral Bilayer Membranes, *J. Am. Chem. Soc.* **107**, 509–510.

Namani, T. and Walde, P.: 2005, From Decanoate Micelles to Decanoic Acid/Dodecylbenzenesulfonate Vesicles, *Langmuir* **21**, 6210–6219.

Naraoka, H., Shimoyama, A. and Harada, K.: 1999, Molecular Distribution of Monocarboxylic Acids in Asuka Carbonaceous Chondrites from Antarctica, *Origins Life Evol. Biosphere* **29**, 187–201.

New, R. P. C.: 1990, *Liposomes, a Practical Approach*, IRL Press, Oxford.

Nielson, P. E.: 1993, Peptide Nucleic Acid (PNA): A Model Structure for the Primordial Genetic Material? *Origins Life Evol. Biosphere* **23**, 323–327.

Nomura, F., Nagata, M., Inaba, T., Hiramatsu, H., Hotani, H. and Takiguchi, K.: 2001, Capabilities of Liposomes for Topological Transformation, *Proc. Natl. Acad. Sci. USA* **98**, 2340–2345.

Nomura, S.-I. M., Tsumoto, K., Hamada, T., Akiyoshi, K., Nakatani, Y. and Yoshikawa, K.: 2003, Gene Expression Within Cell-Sized Lipid Vesicles, *ChemBioChem* **4**, 1172–1175.

Nooner, D. W. and Oró, J.: 1979, Synthesis of Fatty Acids by a Closed System Fischer-Tropsch Process, in E. L. Kugler, F. W. Steffgen, (eds), *Hydrocarbon Synthesis From Carbon Monoxide and Hydrogen*, Am. Chem. Soc., Washington DC, pp. 159–171.

Nowick, J. S. and Chen, J. S.: 1992, Molecular Recognition in Aqueous Micellar Solution: Adenine-Tymine Base-Pairing in SDS Micelles, *J. Am. Chem. Soc.* **114**, 1107–1108.

Oberholzer, T., Wick, R., Luisi, P. L. and Biebricher, C. K.: 1995, Enzymatic RNA Replication in Self-Reproducing Vesicles: An Approach to a Minimal Cell, *Biochem. Biophys. Res. Commun.* **207**, 250–257.

Oberholzer, T., Nierhaus, K. H. and Luisi, P. L.: 1999, Protein Expression in Liposomes, *Biochem. Biophys. Res. Commun.* **261**, 238–241.

Ohkubo, K., Urabe, K., Yamamoto, J., Sagawa, T. and Usui, S.: 1995, Novel Stereoselective Incorporation and Hydrolysis of Long-Chain Amino-Acid Substrates by Vesicular Membrane Systems which Include tri- or Tetra-Peptide Catalysts, *J. Chem. Soc. Perkin Trans.* 1, 2957–2959.

Oparin, A. I.: 1938, *The Origin of Life*, MacMillian, New York.

Oparin, A. I.: 1965, The Origin of Life and the Origin of Enzymes, *Adv. Enzymol.* **27**, 347–380.

Oparin, A. I. and Gladilin, K. L.: 1980, Evolution of Self-Assembly of Probionts, *BioSystems* **12**, 133–145.

Orgel, L.: 1998, The origin of life – a Review of Facts and Speculations, *Trends Biochem. Sci.* **23**, 491–495.

Orgel, L.: 2000, Self-Organizing Biochemical Cycles, *Proc. Natl. Acad. Sci. USA* **97**, 12503–12507.

Orgel, L.: 2003, Some Consequences of the RNA World Hypothesis, *Origins Life Evol. Biosphere* **33**, 211–218.

Oró, J.: 1994, Chemical Synthesis of Lipids and the Origin of Life, *J. Biol. Phys.* **20**, 135–147.

Otto, S. and Engberts, J. B. F. N.: 2000, Diels-Alder Reactions in Water, *Pure Appl. Chem.* **72**, 1365–1372.

Otto, S., Engberts, J. B. F. N. and Kwak, J. C.- T.: 1998, Million-Fold Acceleration of a Diels-Alder Reaction due to Combined Lewis Acid and Micellar Catalysis in Water, *J. Am. Chem. Soc.* **120**, 9517–9525.

Ourisson, G., and Nakatani, Y.: 1994, The Terpenoid Theory of the Origin of Cellular Life: The Evolution of Terpenoids to Cholesterol, *Chem. Biol.* **1**, 11–23.

Paula, S., Volov, A. G., Van Hoek, A. N., Haines, T. H. and Deamer, D. W.: 1996, Permeation of Protons, Potassium Ions, and Small Polar Molecules Through Phospholipid Bilayers as a Function of Membrane Thickness, *Biophys. J.* **70**, 339–348.

Persson, D., Thorén, P. E. G., Esbjörner, E. K., Goksör, M., Lincoln, P. and Nordén, B.: 2004, Vesicle Size-Dependent Translocation of Penetratin Analogs Across Lipid Membranes, *Biochim. Biophys. Acta* **1665**, 142–155.

Podlech, J.: 2001, Origin of Organic Molecules and Biomolecular Homochirality, *Cell. Mol. Life Sci.* **58**, 44–60.

Pohorille, A., Wilson, M. A. and Chipot, C.: 2003, Membrane Peptides and Their Role in Protobiological Evolution, *Origins Life Evol. Biosphere* **33**, 173–197.

Pozzi, G., Biraul, V., Werner, B., Dannenmuller, O., Nakatani, Y., Ourisson, G. and Terakawa, S.: 1996, Single-Chain Polyprenyl Phosphates Form "Primitive" Membranes, *Angew. Chem. Int. Ed. Engl.* **35**, 177–179.

Rasmussen, S., Chen, L., Nilsson, M. and Abe, S.: 2003, Bridging Nonliving and Living Matter, *Artificial Life* **9**, 269–316.

Rasmussen, S., Chen, L., Stadler, B. M. R. and Stadler, P. F.: 2004, Proto-Organism Kinetics: Evolution Dynamics of Lipid Aggregates with Genes and Metabolism, *Origins Life Evol. Biosphere* **34**, 171–180.

Rao, M., Eichberg, J. and Oró, J.: 1982, Synthesis of Phosphatidylcholine Under Possible Primitive Earth Conditions, *J. Mol. Evol.* **18**, 196–202.

Rao, M., Eichberg, J. and Oró, J.: 1987, Synthesis of Phosphatidylethanolamine Under Possible Primitive Earth Conditions, *J. Mol. Evol.* **25**, 1–6.

Raulin-Cerceau, F., Maurel, M.-C. and Schneider, J.; 1998, From Panspermia to Bioastronomy, the Evolution of the Hypothesis of Universal Life, *Origins Life Evol. Biosphere* **28**, 597–612.

Riepe, A., Beier, H. and Gross, H. J.: 1999, Enhancement of RNA Self-Cleavage by Micellar Catalysis, *FEBS Lett.* **457**, 193–199.

Rispens, T. and Engberts, J. B. F. N.: 2001, Efficient Catalysis of a Diels-Alder Reaction by Metallo-Vesicles in Aqueous Solution, *Org. Lett.* **3**, 941–943.

Rispens, T. and Engberts, J. B. F. N.: 2003, A Kinetic Study of 1,3-Dipolar Cycladditions in Micellar Media, *J. Org. Chem.* **68**, 8520–8528.

Rushdi, A. I. and Simoneit, B. R. T.: 2001, Lipid Formation by Aqueous Fischer-Tropsch-Type Synthesis Over a Temperature Range of 100 to 400 °C, *Origins Life Evol. Biosphere* **31**, 103–118.

Russell, M. J. and Hall, A. J.: 1997, The Emergence of Life from Iron Monosulphide Bubbles at a Submarine Hydrothermal Redox and pH Front, *J. Geol. Soc., London* **154**, 377–402.

Sackmann, E.: 1994, Membrane Bending Energy Concept of Vesicle- and Cell-Shapes and Shape-Transitions, *FEBS Lett*. **346**, 3–16.

Sato, Y., Nomura, S.-I. M. and Yoshikawa, K.: 2003, Enhanced Uptake of Giant DNA in Cell-Sized Liposomes, *Chem. Phys. Lett*. **380**, 279–285.

Schlesinger, P. H., Ferdani, R., Pajewski, R., Pajewska, J. and Gokel, G. W.: 2002, A Hydrocarbon Anchored Peptide That Forms a Chloride-Selective Channel in Liposomes, *Chem. Commun*. 840–841.

Schopf, J. W. (ed.): 2002, *Life's Origin: The Beginnings of Biological Evolution*, University of California Press, Berkeley.

Scrimin, P.: 1996, Control of Reactivity in Aggregates of Amphiphilic Molecules, in *Supramolecular Control of Structure and Reactivity*, Perspectives in Supramolecular Chemistry, Vol. 3 (Hamilton A. D., ed.), John Wiley & Sons, Chichester, pp. 101–153.

Scrimin, P., Tecilla, P. and Tonellato, U.: 1994, Chiral Lipophilic Ligands. 1. Enantioselective Cleavage of i-Amino Acid Esters in Metallomicellar Aggregates, *J. Org. Chem*. **59**, 4194–4201.

Segré, D. and Lancet, D.: 2000, Composing Life, *EMBO Reports* **1**, 217–222.

Segré, D., Ben-Eli, D., Deamer, D. W. and Lancet, D.: 2001, The Lipid World, *Origins Life Evol. Biosphere* **31**, 119–145.

Sephton, M. A.: 2002, Organic Compounds in Carbonaceous Meteorites, *Nat. Prod. Rep*. **19**, 292–311.

Shimoyama, A., Naraoka, H., Komiya, M. and Harada, K.: 1989, Analyses of Carboxylic Acids and Hydrocarbons in Antarctic Carbonaceous Chondrites, Yamato-74662 and Yamato-793321, *Geochem. J*. **23**, 181–193.

Simoneit, B. R. T.: 2004, Prebiotic Organic Synthesis under Hydrothermal Conditions: An Overview, *Adv. Space Res*. **33**, 88–94.

Sjöblom, J., Lindberg, R. and Friberg, S. E.: 1996, Microemulsions – Phase Equilibria Characterization, Structures, Applications and Chemical Reactions, *Adv. Colloid Interface Sci*. **95**, 125–287.

Smith, J. Y., Arnold Jr., F. P., Parson, I. and Lee, M. R.: 1999, Biochemical evolution III: Polymerization on Organophilic Silica-Rich Surfaces, Crystal-Chemical Modeling, Formation of First Cells, and Geological Clues, *Proc. Natl. Acad. Sci. USA* **96**, 3479–3485.

Smith, R. and Tanford, C.: 1972, The Critical Micelle Concentration of L-α-Dipalmitoylcholine in Water and in Water/Methanol Solutions, *J. Mol. Biol*.**67**, 75–83.

Soai, K., Shibata, T. and Sato, I.: 2004, Discovery and Development of Asymmetric Autocatalysis, *Bull. Chem. Soc. Jpn*. **77**, 1063–1073.

Stanish, I. and Monbouquette, H. G.: 2001, Engineering A23187/EDTA-Containing Metal-Sorbing Vesicles for Selective Extraction of Divalent Heavy Metal Ions, *J. Membr. Sci*. **192**, 99–113.

Stillwell, W.: 1976, Facilitated Diffusion of Amino Acids Across Biomolecular Lipid Membranes as a Model for Selective Accumulation of Amino Acids in a Primordial Protocell, *BioSystems* **8**, 111–117.

Stillwell, W. and Winter, H. C.: 1973, The Diffusion of Glycine and N-Substituted Glycines Across Bimolecular Lipid Membranes, *Biochem. Biopohys. Res. Commun*. **54**, 1437–1443.

Svetina, S. and Žekš, B.: 2002, Shape Behavior of Lipid Vesicles as the Basis of Some Cellular Processes, *Anat. Rec*. **268**, 215–225.

Szostak, J. W., Bartel, D. P. and Luisi, P. L.: 2001, Synthesizing life, *Nature* **409**, 387–390.

Takakura, K., Toyota, T. and Sugawara, T.: 2003, A Novel System of Self-Reproducing Giant Vesicles, *J. Am. Chem. Soc*. **125**, 8134–8140.

Tanaka, T., Sano, R., Yamashita, Y. and Yamazaki, M.: 2004, Shape Changes and Vesicle Fission of Giant Unilamellar Vesicles of Liquid-Ordered Phase Membrane Induced by Lysophosphatidylcholine, *Langmuir* **20**, 9526–9534.

Taşcioğlu, S.: 1996, Micellar Solutions as Reaction Media, *Tetrahedron* **52**, 11113–11152.

Traïkia, M., Warschawski, D. E., Lambert, O., Rigaud, J.-L. and Devaux, P. F.: 2002, Asymmetrical Membranes and Surface Tension, *Biophys. J*. **83**, 1443–1454.

Trandum, C., Westh, P., Jørgensen, K. and Mouritsen, O. G.: 2000, A Thermodynmaic Study of the Effects of Cholesterol on the Interaction between Liposomes and Ethanol, *Biophys. J.* **78**, 2486–2492.

Trevors, J. T.: 2003, Origin of the First Cells on Earth: A Possible Scenario, *Geomicrobiol. J.* **20**, 175–183.

Treyer, M., Walde, P. and Oberholzer, T.: 2002, Permeability Enhancement of Lipid Vesicles to Nucleotides by Use of Sodium Cholate: Basic Studies and Application to an Enzyme-Catalyzed Reaction Occurring inside the Vesicles, *Langmuir* **18**, 1043–1050.

Tributsch, H., Fiechter, S., Jokisch, D., Rojas-Chapana, J. and Ellmer, K.: 2003, Photoelectrochemical Power, Chemical Energy and Catalytic Acticity for Organic Evolution on Natural Pyrite Interfaces, *Origins Life Evol. Biosphere* **32**, 129–162.

Ueoka, R., Matsumoto, Y., Moss, R. A., Swarup, S., Sugii, A., Harada, K., Kikuchi, J. and Murakami, Y.: 1988, Membrane Matrix for the Hydrolysis of Amino Acid Esters with Marked Enantioselectivity, *J. Am. Chem. Soc.* **110**, 1588–1595.

Ueoka, R., Mori, S. and Moss, R. A.: 1994, Remarkably Enhanced Diastereoselective Hydrolysis by Controlling the Reaction Field, *Langmuir* **10**, 2892–2898.

Umakoshi, H., Shimanouchi, T. and Kuboi, R.: 1998, Selective Separation Process of Proteins Based on the Heat Stress-Induced Translocation Across Phospholipid Membranes, *J. Chromatogr. B* **711**, 111–116.

Vauthey, S., Milo, C., Frossard, P., Garti, N., Leser, M. E. and Watzke, H. J.: 2000, Structures Fluids as Microreactors for Flavor Formation by the Maillard Reaction, *J. Agric. Food Chem.* **48**, 4808–4816.

Velimirov, B.: 2001, Nanobacteria, Ultramicrobacteria and Starvation Forms: A Search for the Smallest Metabolizing Bacterium, *Microbes Environ.* **16**, 67–77.

Vlassov, A., Khvorova, A. and Yarus, M.: 2001, Binding and Disruption of Phospholipid Bilayers by Supramolecular RNA Complexes, *Proc. Natl. Acad. Sci. USA* **98**, 7706–7711.

von Kiedrowski, G.: 2005 (organizer), 1st Workshop on "Systems Chemistry", Venice, October 3–4, 2005.

Wächtershäuser, G.: 1992, Groundworks for an Evolutionary Biochemistry: The Iron-Sulphur World, *Prog. Biophys. Molec. Biol.* **58**, 85–201.

Walde, P.: 2004, Preparation of Vesicles (Liposomes), in *Encyclopedia of Nanoscience and Nanotechnology*, Volume 9. (H. S. Nalwa ed.), American Scientific Publishers, Los Angeles, pp. 43–79.

Walde, P. and Ichikawa, S.: 2001, Enzymes Inside Lipid Vesicles: Preparation, Reactivity and Applications, *Biomol. Eng.* **18**, 143–177.

Walde, P., Wessicken, M., Rädler, U., Berclaz, N., Conde-Frieboes, K. and Luisi, P. L.: 1997, Preparation and Characterization of Vesicles from Mono-*n*-alkyl Phosphates and Phosphonates, *J. Phys. Chem. B*, **101**, 7390–7397.

Walde, P., Wick, R., Fresta, M., Mangone, A. and Luisi, P. L.: 1994, Autopoietic Self-Reproduction of Fatty Acid Vesicles, *J. Am. Chem. Soc.* **16**, 11649–11654.

Walsh, D., Hopwood, J. D. and Mann, S.: 1994, Crystal Tectonics: Construction of Reticulated Calcium Phosphate Frameworks in Bicontinuous Reverse Microemulsions, *Science* **264**, 1576–1578.

Wick, R., Walde, P. and Luisi, P. L.: 1995, Light Microscopic Investigations of the Autocatalytic Self-Reproduction of Giant Vesicles, *J. Am. Chem. Soc.* **117**, 1435–1436.

Wills, C. and Bada, J.: 2000, *The Spark of Life: Darwin and the Primeval Soup*, Oxford University Press, Oxford.

Woese, C.: 1998, The Universal Aancestor, *Proc. Natl. Acad. Sci. USA* **95**, 6854–6859.

Yanagawa, H., Ogawa, Y., Furuta, H. and Tsuno, K., 1989, Spontaneous Formation of Superhelical Strands, *J. Am. Chem. Soc.* **111**, 4567–4570.

Yatcilla, M. T., Herrington, K. L., Brasher, L. L., Kaler, E. W., Chiruvolu, S. and Zasadzinski, J. A.: 1996, Phase Behavior of Aqueous Mixtures of Cetyltrimethylammonium Bromide (CTAB) and Sodium Octyl Sulfate (SOS), *J. Phys. Chem.* **100**, 5874–5879.

Yatsimirsky, A. K.: 2004, Enzyme Mimics, in *Encyclopedia of Supramolecular Chemistry*, (Atwood J. L., Steed J. W., eds.) Marcel Dekker, Inc., New York, pp. 546–553.

Yoshimoto, M. and Kuboi, R.: 1999, Oxidative Refolding of Denatured/Reduced Lysozyme Utilizing the Chaperone-Like Function of Liposomes and Immobilized Liposome Chromatography, *Biotechnol. Prog.* **15**, 480–487.

Yoshimoto, M., Wang, S., Fukunaga, K., Treyer, M., Walde, P., Kuboi, R. and Nakao, K.: 2004, Enhancement of Apparent Substrate Selectivity of Proteinase K Encapsulated in Liposomes Through a Cholate-Induced Alteration of the Bilayer Permeability, *Biotechnol. Bioeng.* **85**, 222–233.

Zemb, T., Dubois, M., Demé, B. and Gulik-Krzywicki, T.: 1999, Self-Assembly of Flat Nanodiscs in Salt-Free Catanionic Surfactant Solutions, *Science* **283**, 816–819.

Zubay, G.: 1996, *Origins of Life on the Earth and in the Cosmos*, Wm. C. Brown Publishers, Dubuque, IA, USA.

Zurbriggen, R.: 2003, Immunostimulating Reconstituted Influenza Virosomes, *Vaccine* **21**, 921–924.