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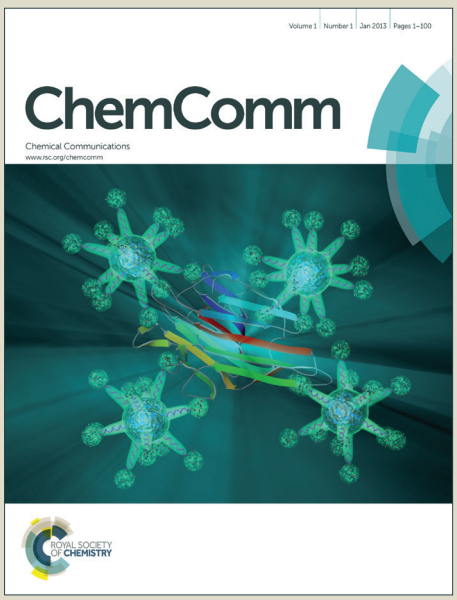

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ARTICLE TYPE

Emergent properties arising from the assembly of amphiphiles. Artificial vesicle membranes as reaction promoters and regulators

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This article deals with artificial vesicles and their membranes as reaction promoters and regulators. Among the various molecular assemblies which can form in an aqueous medium from amphiphilic molecules, vesicle systems are unique. Vesicles compartmentalize the aqueous solution in which they exist, independent on whether the vesicles are biological vesicles (existing in living systems) or whether they are artificial vesicles (formed *in vitro* from natural or synthetic amphiphiles). After the formation of artificial vesicles, their aqueous interior (the endovesicular volume) may become – or may be made – chemically different from the external medium (the exovesicular solution), depending on how the vesicles are prepared. The existence of differences between endo- and exovesicular composition is one of the features on the basis of which biological vesicles contribute to the complex functioning of living organisms. Furthermore, artificial vesicles can be formed from mixtures of amphiphiles in such a way that the vesicle membranes become molecularly, compositionally and organizationally highly complex, similarly to the lipidic matrix of biological membranes. All the various properties of artificial vesicles as membranous compartment systems emerge from molecular assembly as these properties are not present in the individual molecules the system is composed of. One particular emergent property of vesicle membranes is their possible functioning as promoters and regulators of chemical reactions caused by the localization of reaction components, and possibly catalysts, within or on the surface of the membranes. This specific feature is reviewed and highlighted with a few selected examples which range from the promotion of decarboxylation reactions, the selective binding of DNA or RNA to suitable vesicle membranes, and the reactivation of fragmented enzymes to the regulation of the enzymatic synthesis of polymers. Such type of emergent properties of vesicle membranes may have been important for the prebiological evolution of protocells, the hypothetical compartment systems preceding the first cells in those chemical and physico-chemical processes that led to the origin of life.

1 Vesicle types, their general characteristics and emergent properties

1.1 Vesicles as molecular assemblies

The term *vesicle* (from the latin "vesicula", meaning "small bladder") is used in biology, chemistry and pharmacology for a specific type of molecular assembly. Vesicles are formed from amphiphiles[1] in an aqueous medium and contain in their interior an aqueous volume which is separated from the exterior aqueous solution by one or several closed membranous shells (lamellae) of usually thousands of amphiphiles (Fig. 1).[2] The shells define the vesicle's boundary which may be composed of only one or of a few types of amphiphiles, or it may be constituted of a complex mixture of molecules. Depending on the type of vesicles, the amphiphilic molecules forming the shells may be naturally occurring or fully synthetic and they may be of low molar mass or macromolecules, *e.g.* proteins, polypeptides or synthetic block copolymers. Vesicles often are spherical but they may also be non-spherical, *e.g.* tubular, and their sizes may vary from about twenty nanometers to more than hundred micrometers.[3-14]

1. 2 Biological vesicles

In biological systems vesicles play several important roles,[15] for examples (i) in processes which internalize molecules and particles in eukaryotic cells (formation of endosomes),[16] (ii) for the eukaryotic intracellular enzymatic degradation of macromolecules (lysosomes),[17, 18] (iii) for transporting molecules within eukaryotic cells (transport vesicles),[19] (iv) for bringing molecules out of cells (secretory vesicles), or (iv) for the exchange of molecules between cells (extracellular vesicles, *i.e.* exosomes and microvesicles).[20-22] All these types of vesicles are unilamellar and known as *biological vesicles* (Fig. 1A). They contribute in various ways to the functioning of contemporary cells and of "cell communities", whereby one general characteristic of the vesicles is the separation of their entrapped water soluble molecules from the aqueous medium in which the vesicles exist. Therefore, the particular vesicle structure allows

having an endovesicular aqueous volume which is different from the exovesicular environment. One example is the "redox compartmentalization"[23] of endosomes and exosomes. In the reducing cytoplasm of eukaryotic cells endosomes have an oxidizing internal space,[24] while exosomes with their cytoplasmatic reducing interior exist in an oxidizing extracellular medium.

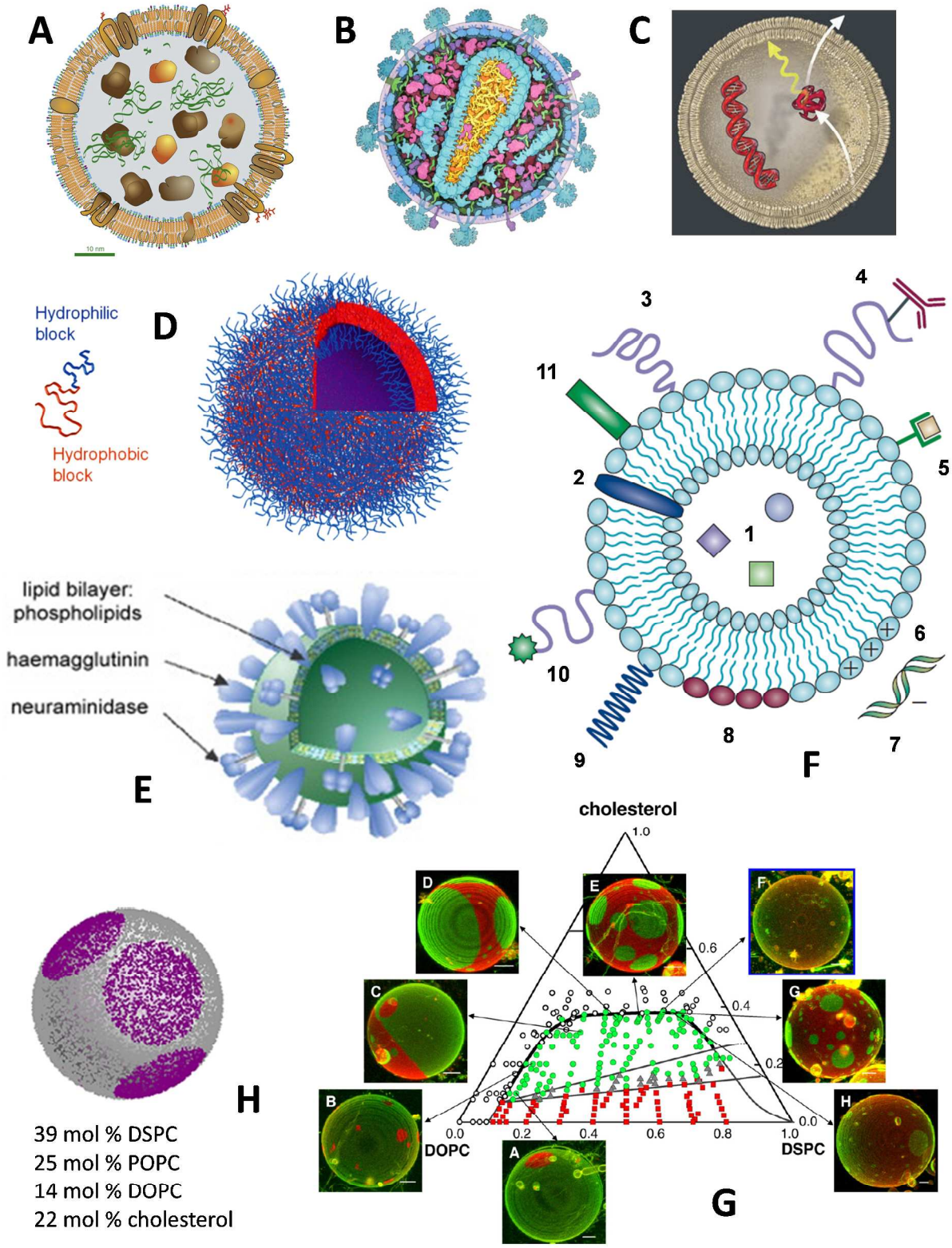


Fig. 1 Schematic representations of different types of vesicular aggregates.

A. Exosome, as an example of a biological vesicle, illustrating that this type of vesicle contains encapsulated cargo proteins (blobs) and RNAs (ribbons), and that the vesicle membrane is composed of a mixture of different types of low molar mass amphiphiles (lipids) and of transmembrane proteins with bound polysaccharide chains which face the exterior. The lipids constituting the membrane are ceramides, sphingomyelins, phosphatidylserines, phosphatidylethanolamines, phosphatidylcholines, lyso-phosphatidylcholines, phosphatidylinositols and cholesterol (as determined for mast-cell derived exosomes). From Vlassov *et al.*[20] **B**. HIV (human immunodeficiency virus), constituted by two strands of RNA, 15 types of viral proteins, and a few proteins from the last host cell the virus infected. The boundary of the shell is formed by a lipid bilayer membrane containing envelope proteins. From RCSB PDP-101 (http://www.rcsb.org/, accessed Feb. 18, 2014). **C**. Model of a vesicular protocell constituted of a membrane boundary of chemically simple amphiphiles and entrapping a double stranded and a folded RNA (ribozyme, in analogy to amino acid-based enzymes). The ribozyme catalyzes metabolic reactions which promote the uptake of nutrients from the environment. From Ricardo and Szostak.[42] **D**. Artificial vesicle formed from a synthetic diblock copolymer. From Lo Presti *et al.*[51] Such "block copolymer vesicles"[50] may also be obtained from amphiphilic polymeptides.[10] **E**. Reconstituted influenza virus envelopes without inner core and genetic information ("Influenza Virosome"), mimicking native influenza viruses. From Herzog *et al.*[65] **F**. The fascinating possibilities for engineering artificial vesicles as multifunctional drug delivery systems. Vesicle-entrapped water-soluble molecules or particles (**1**); membrane-embedded water-insoluble molecule (**2**); lipid-bound hydrophilic polymer (*e.g.* polyethyleneglycol, PEG) for sterical stabilization (**3**); antibody bound to a polymer (**4**) for targeting; lipid with head group bound ligand for the specific non-covalently binding of ions for diagnostic applications (**5**); incorporation of positively charged lipids (**6**) for allowing the binding of negatively charged DNA on the surface of the vesicles (**7**); incorporation of stimuli-responsive amphiphiles (**8**) or attachment of a stimuli-responsive polymer (**9**) for a controlled release within the target cells; attachment of viral components (**10**) or cell-penetrating peptides (**11**) for efficient cell uptake. From Torchilin.[70] **G**. Confocal fluorescence microscopy images of micrometer-sized giant unilamellar vesicles (GUVs) prepared from different mixtures of DOPC, DSPC and cholesterol in presence of two fluorescent dyes which selectively stain either the L_α (l_d)-phase (green) or the L_β (s_o)-phase (red), illustrating coexistence of different microscopic domains within the membrane of the same vesicle, as obtained after equilibration and observation at 23 °C. Scale bar: 5 µm. Based on such measurements the thermodynamic 3-component phase diagram was constructed. From Zhao *et al.*[83] **H.** Illustration of nanoscopic domains present in the membranes of submicrometer-sized vesicles (diameter 60 nm), prepared from a mixture of DSPC (39 mol %), POPC (25%), DOPC (14 mol %) and cholesterol (22 mol %) and analyzed at 20 °C by small angle neutron scattering (SANS) measurements, followed by modeling of the SANS profiles with a Monte Carlo method. The pink color represents the domains. There seems to be a thickness mismatch between the two coexisting phases. From Heberle *et al.*[84]

In addition to the importance of the physico-chemical properties of the *interior* of biological vesicles, as compared to the exterior, the complex composition of the *membranes* of biological vesicles is equally important and therefore worth emphasizing. The vesicle membrane controls the exchange of molecules between the vesicles' interior and the external medium, and it determines the interaction with other membranous structures within a cell. One example is the fusion of secretory vesicles with the plasma membrane to initiate the release of entrapped molecules from the vesicles. This fusion event is "under control" of the membrane components.

With this very general and also rather simple view of (biological) vesicles as membranous compartment systems, certain types of virus particles can be considered as vesicles as well, for example the influenza virus or the human immunodeficiency virus (HIV), see Fig. 1B. Both are a kind of functionalized vesicle with a size in the range of about 100 nm. Viruses themselves are not living entities but they are part of the "living world", usually coexisting in balance with cellular systems.[25]

Overall, biological vesicles are the result of biological evolution that occurred during hundreds of millions of years. Therefore, it is not surprising that biological vesicles are highly sophisticated and molecularly complex. They exert complex functions in complex cellular systems. High molecular complexity is typical for all forms of life for enabling networks of reactions to occur simultaneously within each cell in a spatially and timely controlled manner and with a regulated exchange of matter and energy between cells or between cells and the non-cellular environment.

Since biological vesicles are such essential entities, probably in all forms of contemporary life, it is likely that the concept of vesicular compartments was "invented" by Nature very early in the history of life. Actually, if one takes into account the overall characteristics of a vesicle as closed, membranous compartment structure, one may consider each entire cell as a kind of unilamellar vesicle with complex chemical composition and organization, irrespective of whether the cell is eukaryotic or prokaryotic. The plasma membrane, which defines the boundary of all cells, would be the vesicle shell, and the cell's crowded viscous interior with its DNA, ribosomes and many more biomolecules, with or without subdivisions into organelles, would be the vesicle's aqueous internal space. With this simplified view of cells as sophisticated vesicular compartments and with the fact that all known forms of life are cellular, it is logical to assume that the formation of vesicular compartments on the early Earth was an important step in the processes which led to a transformation of non-living forms of matter into the first living cellular systems, *i.e.* the origin of life about $3.5–3.8 \cdot 10^9$ years ago.[26-28] The prebiological appearance of cell-like compartments probably was equally important as the formation of nucleotides and RNA or RNA-like macromolecules. One may even argue that the formation of vesicular compartments in prebiotic times probably was much easier than the synthesis of functional oligomers or polymers.[29] Indeed, many studies have shown that vesicles form easily in aqueous solution *in vitro* not only from many different types of amphiphiles, but in particular also from chemically very simple ones, even from small amphiphiles with only one functional group, *e.g.* from polyprenyl phosphates, nonanoic or decanoic acid.[30-35] Such amphiphiles may have been present on Earth before life existed.[34, 36, 37] Therefore, one can assume that the formation of vesicles (and other types of assemblies)[32] occurred in prebiotic times from prebiotic amphiphiles. This is the reason why vesicles prepared from potentially prebiotic amphiphiles currently are considered as *models of protocells*, the hypothetical precursor structures of the first cells (Fig. 1C).[30, 33, 34, 37-45] In this scenario, it is thought that protocell systems were not yet living but already had many characteristic features of cells, both from a structural as well as from an organizational point of view.

1. 3 Artificial vesicles

As mentioned above and later in section 3, vesicles which are prepared and investigated as models of protocells. These types of vesicles are non-biological since they do not exist in living systems. They are artificial and, therefore, belong to the group of *artificial vesicles*. So-called lipid vesicles (liposomes)[46] are also artificial vesicles. They can be obtained in aqueous solution from individual amphiphiles or mixtures of amphiphiles, imprecisely called 'lipids', isolated from biomembranes. Well known are phospholipid vesicles which are obtained from lipids which bear at least one phosphate group in their chemical structure (Fig. 2).

The formation of phospholipid vesicles *in vitro* was first demonstrated by Bangham and coworkers.[47] They convincingly showed that turbid phospholipid suspensions, which form by dispersing in aqueous solutions phospholipids bearing two hydrophobic chains, contain aggregates which have an aqueous interior volume which is separated from the bulk aqueous medium by several self-closed membranous layers of the phospholipids. After these seminal findings, Gebicki and Hicks[48] demonstrated vesicle formation from naturally occurring unsaturated fatty acids. Later on, Kunitake and Okahata[49] for the first time, showed that vesicle formation is also possible from certain fully synthetic, non-natural amphiphiles, di-*n*-dodecyl- or di-*n*-tetradecyldimethylammonium bromide, abbreviated as $2C_{12}N^{+}2C_1Br$ and $2C_{14}N^{+}2C_1Br$ (Fig. 2). This finding initiated a vast amount of studies with a variety of vesicle forming amphiphiles, including in recent years many different types of amphiphilic block copolymers (Fig. 1D).[8, 9, 11, 50-56]

Multi- or unilamellar artificial vesicles are prepared and investigated for various reasons by many researchers, not only as protocell models or for the construction of artificial cell-like systems.[57-63] Unilamellar artificial vesicles are applied, for example, for vaccination (the vesicles in this case imitate the size and the surface characteristics of the influenza virus, Fig. 1E),[64-66] or they may be used as *drug delivery systems* since hydrophilic as well as lipophilic drugs can be hosted by vesicles, and since the vesicle membrane can be functionalized as desired (Fig. 1F).[67-74]

Common to all these types of functional artificial vesicles is that they are obtained from the chosen amphiphiles on the basis of *molecular self-assembly* in combination with a desired *engineering of the assembly*. The self-assembly of the amphiphiles is the underlying driving force for bringing membrane-forming molecules together so that they form vesicular compartment structures as a consequence of the chemical structure of the amphiphiles and of the experimental conditions, *i.e.* concentration, composition of the aqueous solution, temperature. A desired average vesicle size, lamellarity, or a desired asymmetry of the membranes usually are only obtained if additional engineering steps are included in the preparation, *i.e.* upon applying a certain method of vesicle preparation.[14, 76-78] Therefore, artificial vesicles of a relatively defined size and lamellarity, independent on whether the vesicle membranes are composed of chemically complex or simple amphiphiles, usually are obtained through *"guided assembly"* processes,[79] resulting in vesicle systems which are *not* representing true thermodynamic equilibrium states.[80] From a practical point of view this is an important property and it means that a detailed description of the procedure with which artificial vesicle suspension or individual surface-adsorbed vesicles are prepared (*i.e.* giant vesicles adsorbed onto glass surfaces or metal wires)[14] is of outmost importance.[4, 14, 76-78, 81]

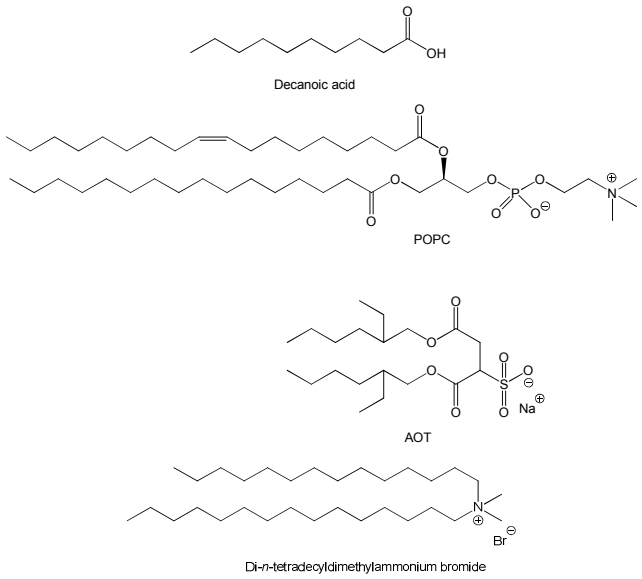


Fig. 2 Chemical structures of four selected amphiphiles from which vesicle formation in an aqueous medium is observed experimentally at room temperature. Decanoic acid: vesicles form if about half of the molecules are ionized (deprotonated).[222,223] POPC (1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine): one of the most intensively investigated vesicle-forming phospholipid.[3,78] **AOT** (sodium bis(2-ethylhexyl)sulfosuccinate): vesicles form in the presence of salt.[224,225] Di-*n*-tetradecyldimethylammonium bromide (*N,N*-ditetradecyl-*N,N*-dimethylammonium bromide, $2C_{14}N^{+}2C_1Br^{-}$): one of the first synthetic amphiphile from which vesicle formation has been demonstrated.[226]

Beyond the well-known applications as drug delivery systems or as protocell models, artificial vesicles often are also used as *biomembrane-mimicking model systems.* In this case, the vesicles are prepared for studying certain biophysical properties of biomembrane lipids in their assembled state, thereby assuming that the *in vitro* behavior of assembled biomembrane amphiphiles reflects the behavior of the same molecules within native membranes.[82] Examples are (i) the non-homogeneous mixing of biomembrane lipids if a mixture of different types of lipids is used, *i.e.* the formation of microscopic domains (Fig. 1G)[83] or of nanoscopic rafts (Fig. 1H),[84] or (ii) the analysis of membranes formed from one type of lipid at a temperature which is at or close to the melting temperature of the lipids (coexistence of patches of "gel-like" (solid-ordered, abbreviated as s_o or L_β) and fluid (liquid-disordered, l_d or L_α) states).[85] In these two examples, the vesicles investigated often are prepared in such a way that the arrangement of the lipids in membranes of individual vesicles represents lowest free energy states, which then allows drawing thermodynamic phase diagrams.[83, 84, 86-91] In these investigations, the composition of the aqueous interior of the vesicles and of the exterior bulk solution certainly may have an influence on the phase behavior of the lipids within the membrane. The main interest with these types of studies, however, lies entirely in a

description of the membrane boundary of the vesicles, as it is obtained on the basis of inter- and intra-molecular interactions and molecular motions if enough time is given for the system to equilibrate (true self-assembly state).[92]

1. 4 Common characteristics and emergent properties of artificial vesicles

Considering the above-mentioned structural features of individual artificial vesicles and also taking into account the reactivity of entire vesicle systems (*i.e.* volumes composed of a number of vesicle compartments), it is possible to identify four main properties of all types of *artificial vesicle systems*:

(I) the size and composition of the vesicles' aqueous internal (endovesicular) volume, which may be chemically different from the external (exovesicular) medium;

(II) the composition and physical state of the vesicles' membranes

(III) the morphology of the vesicles (lamellarity and shape);

(IV) the transformation of the vesicles in terms of (i) changes of the composition of the aqueous interior of the vesicles, (ii) changes of the composition of the membranes of the vesicles, (iii) changes of the vesicle size and morphology, and (iv) changes of the vesicle number, as a result either of physical forces acting on the system, of membrane-forming amphiphiles added to the exovesicular volume, or of chemical reactions taking place in the system (Fig. 3).

Properties (I) to (III) are descriptions of the vesicles as *"individual objects"* and are mainly based on their molecular composition and physical state. Importantly, these properties can be seen as stationary properties - mainly determined by the preparation procedure – that are not perturbed by the dynamics of the molecules constituting the vesicles, *e.g.* by the mobility of the amphiphiles within the vesicle membranes or the exchange with the endo-and exovesicular volumes (Fig. 3A).

On the other hand, property (IV) captures the behavior of the vesicles considered as *dynamical system*, a system composed of many vesicular compartments which can undergo chemical and physico-chemical changes due to a coupling with chemical reactions, uptake of amphiphiles from the environment, or due to unbalanced physical forces (osmotic pressure or mechanical perturbations) that may act on the system (Fig. 3B).[93-101] All the schematic drawings in Fig. 1 and Fig. 3A are simplified illustrations of snapshots of (average) structures of individual vesicles only, representing, however, experimental findings. Fig. 3B illustrates changes that may occur in vesicle systems with time.

Due to an intrinsic vesicle "diversity" which is generated by microscopic local conditions and molecular stochastic processes at the moment of vesicle formation, all populations of artificial vesicles are heterogeneous with respect to properties (I) to (III) (and, consequently, with respect to property (IV)). This structural heterogeneity can be reduced to some extent during the vesicle formation process but cannot be eliminated completely. Therefore, from a theoretical point of view, for a description of the time dependent behavior of chemically reacting artificial vesicle systems, one has to take into account the role of random fluctuations[102-106] that may become highly important if the vesicle size is small with only a small number of reacting molecules in each compartment (intrinsic stochasticity effect).[107, 108] Moreover, the vesicle preparation method used may produce a population of artificial vesicles in which the vesicles may differ from each other considerably, both with respect to size and composition of the internal volume (extrinsic stochasticity effect).[109, 110] This may give rise to a population of individual vesicles which exhibit very different internal component concentrations ("spontaneous crowding")[111, 112] and very different time behaviors.

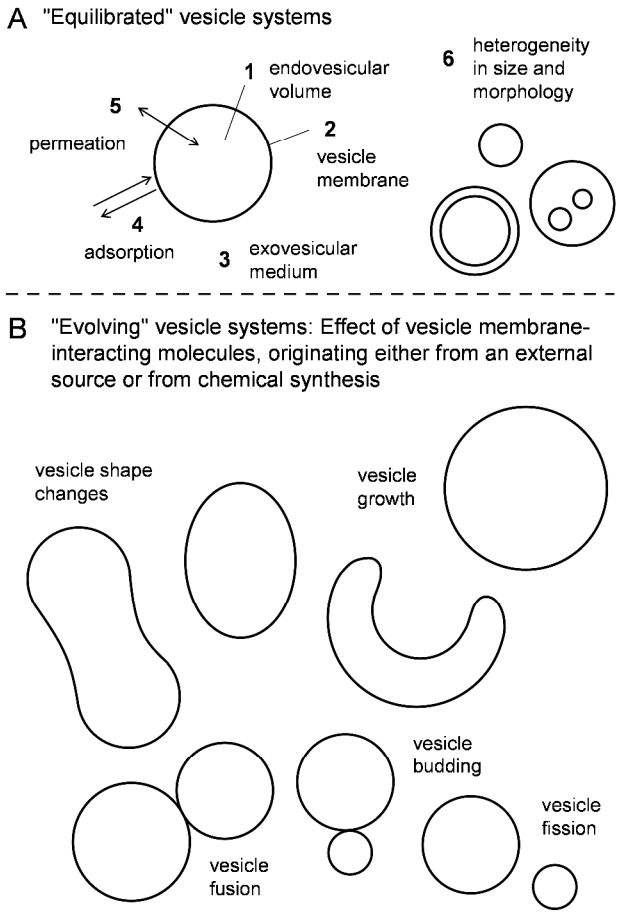


Fig. 3 A. Schematic representation of a vesicle system containing individual vesicles as "objects". Characteristic features are: 1, the size and composition of the vesicle's interior volume (endovesicular space); 2, the vesicle membrane (lamellarity, molecular composition, dynamics and organization); 3, the external medium in which the vesicles are dispersed (exovesicular space); 4, the binding properties of the vesicle membrane (solute dependent adsorption); 5, the exchange of molecules between the external and internal spaces (solute dependent membrane permeation); and 6 the heterogeneity of the vesicle system with respect to size and morphology (uni-, oligo- or multilamellar vesicles, smaller vesicles with a larger vesicle). **B**. Due to chemical and physical processes (*e.g.* induced osmotic pressure differences between the endo- and exovesicular spaces), the vesicles may undergo morphological changes (increase or decrease in vesicle size, vesicle shape changes, vesicle budding and fission, vesicle fusion).[13, 227, 228]

The formation of vesicle systems leads to new properties which are neither present in the non-assembled amphiphiles alone, nor in the solution in which the assembly takes place.

These new properties emerge from the assembly process. This is true for all types of vesicle-forming processes, *i.e.* for vesicles obtained through pure self-assembly[113-116] – which also includes chemical reactions leading to a transformation of non-associating or micelle-forming molecules into vesicle-forming amphiphiles[117-120] – or through a guided assembly of amphiphilic molecules.

If we ignore for the obtained vesicles systems any chemical and physical processes which may lead to changes in size, shape and number of vesicles (property (IV) discussed above, Fig. 3B), then the *emergent properties* originating from the formation of the vesicles[121] can be grouped according to the three characteristic features of vesicles as individual objects, as mentioned above: the formed vesicle interior (emergent effects arising from compartmentalization; property I), the formed vesicle membrane (emergent effects arising from the membraneous state of the amphiphiles; property II), and the obtained vesicle morphology (emergent effects arising from a particualr size and shape of the vesicles; property III).

In this article, we focus on some specific *emergent properties of artificial vesicle membranes*, only.[122] With a few selected examples we put these properties in the context of possible applications as soft reaction interfaces. The examples given should illustrate the general concept and should serve as motivation for a further more in depth exploration of vesicles as reaction "additives", *i.e.* as dispersed soft, functional interfaces, for influencing chemical reactions in a desired way. Finally, the possible role of vesicles for the prebiological formation of complex molecules on the surface of the vesicles will be briefly discussed, recalling a scenario which originally was proposed by Blobel in 1980.[123-125] The idea is that complex molecules may have been formed on the external surface of prebiological vesicles as a result of the emergent properties of the vesicle membranes. Subsequently, these molecules may have become integrated within the interior of the vesicles as a result of physico-chemical processes which may have involved morphological changes of the vesicles (Fig. 3B), possibly even caused by the particular molecules formed on the external vesicle surface.[123-125] This idea compares with the more classical view of the role of prebiological compartments where molecules are entrapped inside vesicles and exert their function, for example as catalyst, from inside of the vesicles.[121]

2 Artificial vesicle membranes as reaction "promoters" and "regulators"

2. 1 General concepts

Any type of assembly of amphiphiles may promote or inhibit chemical reactions due to a localization of reacting molecules within the hydrophobic region of the assembly or on the assembly surface. Due to this localization, the kinetics and pathways of chemical reactions may be altered on the basis of at least three effects, (i) the increased concentration of the reacting species in the area of the assembly, (ii) a different polarity of the actual locus where the reaction takes place, and (iii) steric hindrance for some reactions to take place.

The effect of molecular assemblies on chemical reactions is well known for micellar aggregates under the term "micellar catalysis",[126-131] although the micelles cannot be considered true catalyst since they can also affect the final equilibrium state. Similarly to micelles, the effect of artificial vesicles on chemical reactions is also known since many years.[132-142] However, since it is somewhat more difficult to work with vesicle systems as compared to micelles (as outlined above),[143] the effect of vesicles as reaction "promoters" and "regulators" is much less explored. Important early contributions were from the groups of Kunitake,[144, 145] Murakami,[146-148] Moss,[147, 149] Groves,[150, 151] and Nolte,[152] as comprehensively and competently discussed previously by Scrimin.[134] With a few selected examples some of the early work in this field will be mentioned, together with highlights of the more recent developments. The examples mentioned are by no means a complete list. They only serve to illustrate some of the experimental approaches undertaken and the general lines of thoughts. Obviously, there is one prerequisite for all examples: there must be an interaction between the reacting species and the vesicle membrane to allow the "promotion" or "regulation" of reactions by the vesicle membrane.

2. 2 Effect of vesicle membrane microenvironment and fluidity (unimolecular reactions)

One early example is the study of the decarboxylation of 6-nitrobenzisoxazole-3-carboxylate, in the presence of vesicles formed from dialkyldimethylammonium bromides, *e.g.* from di-*n*- tetradecyldimethylammonium bromide (Fig. 2) at pH = 9.1.[145] This unimolecular reaction is shown in Fig. 4; it is known to be dependent on the polarity of the solvent and to be accelerated in the presence of cationic micelles.[132, 153] Studies with the vesicles showed that the reaction is accelerated due to a different microenvironment on the surface of the vesicles as compared to the exovesicular medium.[145] Furthermore, the fluidity of the vesicle membrane has an influence on the reaction rate, the reaction being slower in the s_o-state of the membrane where the amphiphiles are densely packed and hinder efficient binding of 6-nitrobenzisoxazole-3-carboxylate; in the fluid l_d-state, above the chain melting temperature, T_m, the reaction is faster than below T_m, with a clear discontinuity in the behavior occurring in the region of T_m.[145] With this classical example, two possible features of vesicle membranes as reaction promoters are illustrated, (i) the localization of the reaction in an environment which is different from the bulk medium, and (ii) the significant influence of the physical state of the vesicle membrane which influences the surface properties of the membrane. By using vesicles prepared from a mixture of di-*n*-octadeyldimethylammonium bromide and 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) at pH = 8.1, the rate of decarboxylation of 6-nitrobenzisoxazole-3-carboxylate under the experimental conditions used was found to be particularly high if the content of DMPC was 30 mol %.[154] Furthermore, it was shown that the presence of additives, either localized within the membranes of cationic amphiphiles (*e.g.* cholesterol) or on the membrane surface (trehalose) may have an influence of the rate of decarboxylation of 6-nitrobenzisoxazole-3-carboxylate,[155] the reaction being inhibited in presence of cholesterol and accelerated in presence of trehalose, if analyzed

under the particular conditions used.[155] Experiments also indicate that the surface dynamics of the vesicle membranes is an important factor for efficiently catalyzing the reaction (possibly through an influence of the solvation of the substrate and reaction intermediates).[156] All this indicates that small changes in the physical state of the vesicle membrane can have significant effects on the membrane's ability as reaction promoter. As mentioned above, a prerequisite for a reaction to occur localized on the vesicle membrane (or within the membrane) is the binding of the reacting molecules to the vesicles. This is fulfilled in the example of Fig. 4, *i.e.* the binding of the negatively charged 6-nitrobenzisoxazole-3-carboxylate to the positively charged vesicles. Using negatively instead of positively charged vesicles, the decarboxylation of 6-nitrobenzisoxazole-3-carboxylate was either unaffected or retarded.[132]

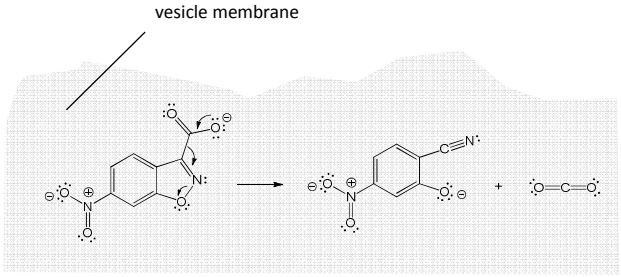


Fig. 4 Schematic representation of the decarboxylation of 6-nitrobenzisoxazole-3-carboxylate occurring in a vesicle membrane. The grey background represents a vesicle membrane which contains di-*n*-tetradecyldimethyl ammonium bromide amphiphiles. Under optimal conditions, the reaction is accelerated in the presence of the vesicles due to a localization of the reaction in the region of the vesicle membrane. Molecular details for convincingly explaining the accelerating effect of the vesicle membrane on the reaction are not know, although it must be the vesicle membrane's microenvironment which promotes the reaction since the microenvironment is different from the exovesicular medium.[145, 154-156]

2. 3 Co-localization of reactants on or within vesicle membranes (bimolecular reactions)

In a study related to the vesicle-promoted decarboxylation of 6-nitrobenzisoxazole-3-carboxylate,[145] the group of Engberts investigated the effect of cationic vesicles at pH ≈ 11.4 on the deprotonation of 5-nitrobenzisoxazole, leading to an elimination of one molecule of water (Kemp elimination), and found an acceleration of the reaction rate by the vesicles (Fig. 5).[157] Vesicles from di-*n*-dodecyldimethylammonium bromide were particularly effective for this bimolecular reaction which again is known to be solvent polarity dependent.[157] Main reasons for the increased rate of elimination, as compared to the reaction in bulk aqueous medium, appear to be (i) the increased local concentration of the two reacting species (5-nitrobenzisoxazole and the hydroxide ion), (ii) the lower dielectric constant on the surface of the vesicles (leading to less strongly hydrated hydroxide ions), (iii) the lower micropolarity, and (iv) the fluidity of the vesicles membrane (smaller effect observed with similar bilayers which are in the s_o-state, *i.e.* below T_m).[157] If mixed vesicles prepared from positively and negatively charged amphiphiles were used, di-*n*-octadecyldimethylammonium bromide and sodium didecyl phosphate (partially forming neutral domains in the vesicle membrane, depending on the relative amounts of the two amphiphiles), the accelerating effect of the vesicles for the deprotonation reaction decreased with increasing content of the anionic component, indicating the importance of the binding of the hydroxide ions to the vesicle surface.[158] Addition of oleoyl alcohol or *n*-dodecyl-β-glucoside or *n*-dodecyl-β-maltoside to cationic vesicles formed from di-*n*-octadecyldimethylammonium bromide led to an increase in the efficiency of the mixed vesicles for this particular elimination reaction, while addition of *n*-decanol or *n*-octadecanol had the opposite effect.[159] As pointed out by the authors of this work,[159] "subtle changes in the structure of the additive can lead to significant changes in the interfacial structure of the vesicles. However, these changes do not originate from a change in polarity, indicating that other factors (*e.g.* water concentration, bilayer packing, domain formation *etc.*) play a more important role."[159]

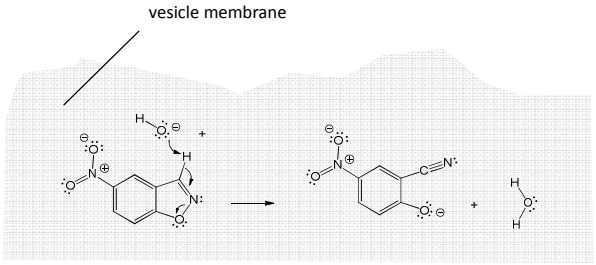


Fig. 5 Schematic representation of the deprotonation of 5-nitrobenzisoxazole (Kemp elimination) occurring in a vesicle membrane. The grey background represents a vesicle membrane which contains di-*n*-dodecyldimethyl ammonium bromide amphiphiles. Under optimal conditions, the reaction is accelerated in the presence of the vesicles due to a co-localization of both reacting species, 5-nitrobenzisoxazole and the hydroxide ion, in the region of the vesicle membrane.[157-159]

2. 4 Co-localization of reactants and organic catalysts on or within vesicle membranes

Since the vesicle membrane can host organic molecules as well as inorganic ions, either embedded within the membrane together with an organic molecule as ligand to form an organic-inorganic complex, or associated on the membrane surface, these added molecules and ions may contribute to a chemical reaction significantly, not only by direct involvement in the reaction, but also indirectly as catalysts. This is illustrated with the following examples. Again, as a general feature of the systems, a co-localization of chemical species within or on the surface of the vesicle membrane is essential for a particular reaction to be promoted or regulated by the membrane. Furthermore, the different polarity within or on the surface of the vesicle membrane, as compared to the exovesicular medium, may contribute to the reaction.

In extensive studies carried out by Ueoka, Ohkubo and collaborators,[147, 160-164] the effect of vesicle-membrane-embedded short peptides on the rate of hydrolysis of membrane-bound activated amino acid esters was investigated, as summarized by Davie *et al.*[165] Using as vesicle-forming amphiphile di-*n*-tetradecyldimethylammonium bromide, vesicle membrane-embedded Z-L-Leu-L-His-OH or Z-L-Phe-L-His-L-Leu-OH

catalyzed the hydrolysis of *p*-nitrophenyl *N*-dodecanoyl-phenylalanylate at pH ≈ 7.7 enantioselectively (Fig. 6A).[147] The L-enantiomer of the ester reacted faster than the D-enantiomer.[147, 164] Based on ^{1}H NMR measurements[162] (and molecular dynamics simulations[166]) a model was presented for explaining the observed enantioselectivity which originates from (i) an optimal conformation of the peptide catalysts within the vesicle membrane,[147, 163] and (ii) an optimal interaction between the membrane-embedded L-enantiomer of the substrate and the peptide catalyst (Fig. 6B).[162] Therefore, the vesicle membrane promotes the reaction by two factors, the alteration of the conformation of the peptides to become (better) catalysts and the co-localization of these peptide catalysts and the substrate. Using slightly different peptide catalysts with a different hydrophobic protecting group at the N-terminus, *i.e. N*-(N^{α}-dodecanoyl-L-His)-L-Leu-OH, it could be shown that similar enantioselectivities in the hydrolysis of membrane-bound activated amino acid esters can be achieved.[167, 168] The presence of cholesterol in the vesicle membrane had an influence on the enantioselectivity which could be correlated with a change in the membrane fluidity.[169] From a pure chemical point of view it is obvious that the presence of His in the peptide structure is important as it is the imidazole ring of the His side chain which acts as an acid-base catalyst, in analogy to the many cases where His residues are at the active site of enzymes.[170] However, it is the hydrophobicity of the peptides used − originating from the hydrophobic protecting group or the other amino acids present in the peptides (Leu or Phe) – which help positioning the His residue within the vesicle membrane.

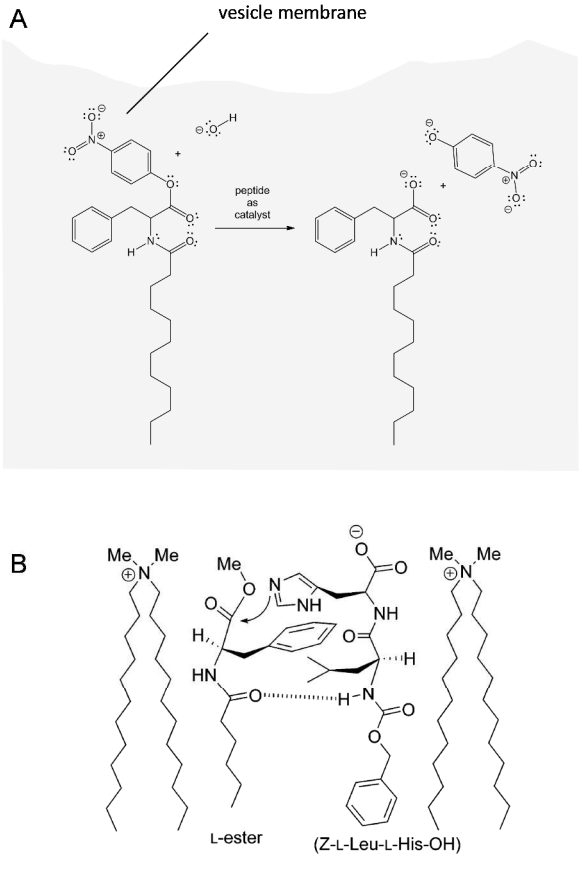


Fig. 6 Schematic representations of a peptide catalyzed hydrolysis of an activated amino acid derivative in a vesicle membrane.[147] **A.** A racemic mixture of the amino acid ester *p*-nitrophenyl *N*-dodecanoyl-phenylalanylate is hydrolyzed at slightly alkaline pH within the membranes of vesicles formed from di-*n*-tetradecyldimethylammonium bromide. The reaction is catalyzed by membrane-embedded hydrophobic peptides, whereby the hydrolysis occurring enantioselectively with either Z-L-Leu-L-His-OH or with Z-L-Phe-L-His-L-Leu-OH. **B.** Close proximity of the L-enantiomer of the amino acid ester and the peptide catalyst (Z-L-Leu-L-His-OH) within the vesicle membrane explains the observed enantioselectivity, as elaborated from ^{1}H NMR measurements for a (less reactive) methyl ester derivative with a hexanoyl chain as protecting group.[162] Drawing from Davie *et al.*[165]

2. 5 Co-localization of reactants and inorganic (co-)catalysts on or within vesicle membranes

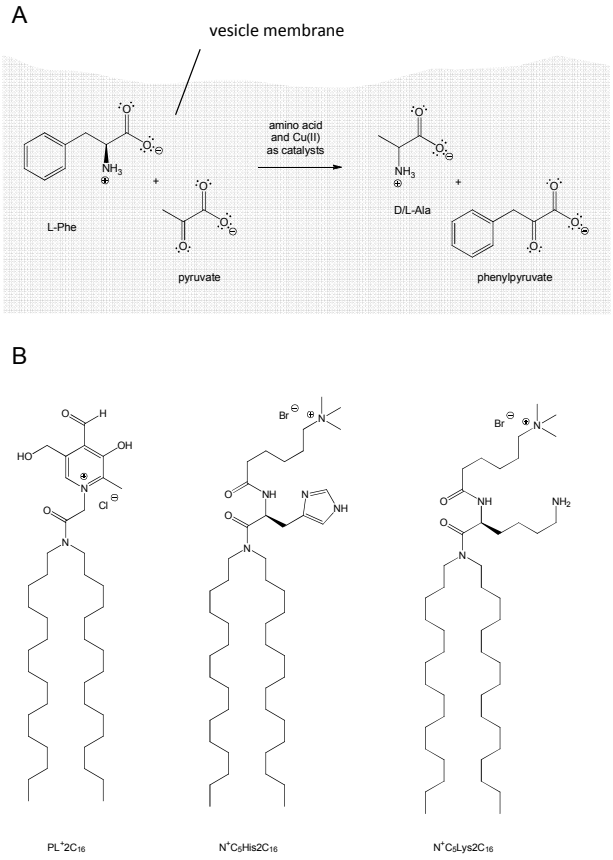
In addition to the embedding of hydrophobic organic molecules (peptides) within vesicle membranes for catalyzing chemical reactions occurring in the region of the membrane (Fig. 6), the complexation of inorganic ions as catalysts (or co-catalysts) in vesicle membranes is also possible, as for example demonstrated by Murakami and coworkers.[133, 135, 146, 148, 171] In this pioneering and very detailed work, the transamination of the hydrophobic amino acid L-Phe with pyruvate, a hydrophilic α-keto acid, was investigated and found to be catalyzed at pH = 5.1 (T = 30 °C) by vesicle membranes composed of a mixture of two cationic amphiphiles, one bearing a pyridoxamine group (abbreviated as PL^+2C_{16}), the other bearing a histidyl residue ($N^+C_5His2C_{16}$), and copper(II) ions as the inorganic ions. The reaction products obtained from L-Phe and pyruvate are Ala (as racemate) and phenylpyruvate, see Fig. 7. This initially studied vesicular system was modified and optimized and showed substrate selectivity (mainly due to differences in the binding of the substrates to the vesicle membrane),[172] high enantioselectivity (if a cationic amphiphile bearing a lysinyl instead of the histidinyl residue was used, $N^+C_5His2C_{16}$)[148] and turnover behavior,[133] like in the case of enzymes. This is why this functionalized vesicle system can be considered as "artificial aminotransferase" mimic.[133, 135, 171, 172] Under optimal reaction conditions, vesicles formed from $N^+C_5Lys2C_{16}$ and PL^+2C_{16} catalyzed with an enatiomeric excess (*ee*) of 90-92 % the transformation of L-Phe and pyruvate into D-Ala and phenylpyruvate at pH = 7.0 and T = 30 °C.[148]

In much less sophisticated vesicle systems, the metal-catalyzed hydrolysis of activated esters occurring on the surface of the vesicles was investigated.[173, 174] Using a lipophilic chiral pyridinyl ligand and cationic vesicles formed from di-*n*-octadecyldimethylammonium bromide, it was shown that the hydrolysis of phenylglycyl-*p*-nitrophenylester (H- Phe-Gly-ONp) at pH = 5.5 occurs with high enantioselectivity (k_D/k_L = 26) if the reaction is carried out in the s_o-state of the vesicle membrane (at T = 2.5 °C), *i.e.* at a temperature much below below T_m ≈ 28 °C.[174]

Phospholipid vesicles containing membrane-spanning metalloporphyrins were investigated by Groves and Neumann[150, 175, 176] and found to act as site-selective catalysts for regioselective oxidation reactions occurring in the core of the membrane. In the case of vesicles prepared from DMPC, the hydroxylation of cholesterol with a steroidal manganese(III) prophyrin derivative in presence of O_2 and ascorbate at pH = 8.6

occurred selectively at C-25 (Fig. 8).

Most of the vesicle membrane-assisted reactions investigated so far and highlighted with a few examples in Figs. 4-8 are oxidative and hydrolytic reactions of small molecules and decarboxylations leading to the degradation of molecules or to small modifications of molecules. Furthermore, often the products diffuse away form the vesicle membrane after the reaction took place. Two additional examples are listed in Table 1.[177, 178] Much less investigated yet are vesicle membrane-promoted or –controlled synthetic reactions which lead from small molecules to larger molecules. Three selected examples are briefly mentioned, a Diels-Alder cycloaddition reaction catalyst be "metallo-vesicles" (Fig. 9),[179] the synthesis of Trp from Ser and indole (Fig. 10),[135, 180, 181] and the synthesis of β-phenylserine from glycine and benzaldehyde (Fig. 11).[182,183]



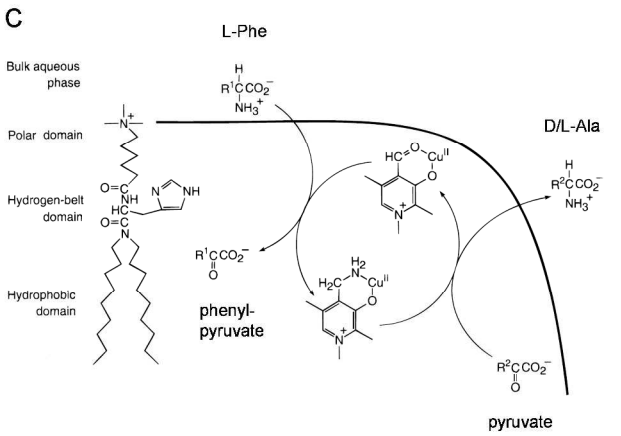


Fig. 7 Schematic representations of a vesicular aminotransferase-mimicking system, as developed by Murakami and collaborators.[133,135,146,148,171] **A.** A mixture of the hydrophobic amino acid L-phenylalanine and the hydrophilic pyruvate is converted at pH = 5.1 into alanine and phenylpyruvate in the presence of vesicles formed from $PL^{+}2C_{16}$ (a hydrophobic pyridoxamine derivative) and $N^{+}C_5His2C_{16}$ in the presence of copper(II) ions. **B.** Chemical structures of $PL^{+}2C_{16}$, $N^{+}C_5His2C_{16}$, and $N^{+}C_5Lys2C_{16}$. **C.** Illustration of the transamination cycle with $N^{+}C_5His2C_{16}$ (left) and $PL^{+}2C_{16}$ complexed to Cu(II) as catalysts (center); R_1: C_6H_5-CH_2-; R_2: CH_3-, adopted from Murakami *et al.*[135]

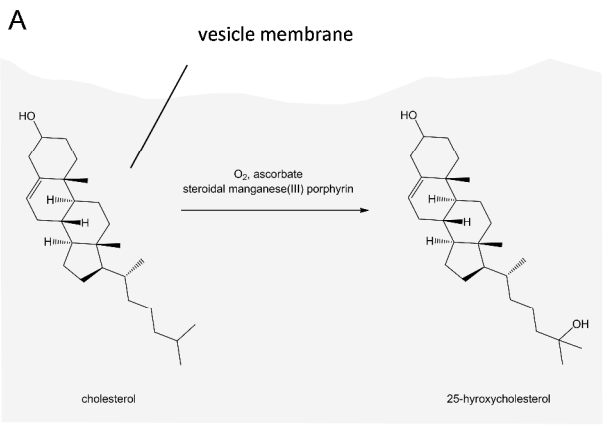


Fig. 8 Schematic representation of the regioselective hydroxylation of cholesterol within the membrane of vesicles formed from DMPC and containing a membrane-spanning steroidal manganese(III) porphyrin at pH =8.6 in the presence of dioxygen and ascorbate. **A.** Overall reaction which converts cholesterol to 25-hydroxycholesterol. **B.** Illustration of the localization of the manganese(III) porphyrin derivative within the DMPC bilayer, drawn together with one molecule of 25-hydroxycholesterol. Drawing from Groves and Neumann.[175]

2. 6 Oligomerization and polymerization reactions on vesicle membranes

Examples for oligomerization or polymerization reactions occurring on the surface of vesicles are listed in Table 2.[184-188] Using either CDI (*N,N'*-carbonyldiimidazole) to activate amino acids (formation of *N*-carboxyanhydride (NCA)-amino acids) or the lipophilic condensing agent EEDQ (*N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline), amino acid oligomerizations and peptide condensation reactions could be achieved on the surface of phospholipid-based vesicles, see Fig. 12.[184, 185] Using cationic vesicles formed from di-*n*-dodecyldimethylammonium bromide and α-thioglutamate (as an activated form of glutamate), amino acid oligomerization on the surface of the vesicles occurred in the presence of bicarbonate buffer, see Fig. 13.[186] Using anionic vesicles formed from AOT (Fig. 2) at pH = 4.3 and T = 25 °C it is possible to polymerize aniline on the surface of the vesicles with the enzyme horseradish peroxidase isoenzyme C (HRPC) as catalyst and hydrogen peroxide (H_2O_2) as oxidant (Fig. 14).[189, 190] The presence of the vesicles has a big influence on the outcome of the reaction, whereby the experimental conditions can be chosen such that the half-oxidized emeraldine salt form of polyaniline can be obtained in high yield. Co-localization of aniline monomers, reaction intermediates and the enzyme catalyst on the vesicle surface is essential for the reaction to proceeding successfully.[189, 190]

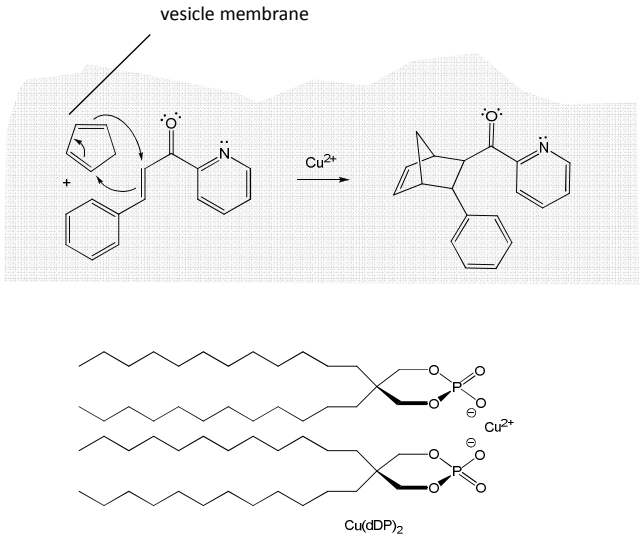


Fig. 9 Schematic representation of a Diels-Alder cycloaddition occurring between cyclpentadiene and the dienophile (*(E)*-3-phenyl-1-(pyridin-2-yl)prop-2-en-1-one) in the membranes of vesicles formed from the metallo-amphiphile Cu(dDP)$_2$ (copper(II) 5,5-di-*n*-dodecyl-1,3,2-dioxaphosphinan-2-olate 2-oxide).[179] The vesicles prepared had an average diameter of about 40 nm and the reaction appears to occur with high efficiency at T = 25 °C due to a binding of cyclopentadiene to the hydrophobic part of the membranes, the binding of the dienophile on the surface of the membrane, and the presence of Cu(II) as Lewis-acid catalyst.[179]

In all these examples of vesicle membrane-assisted oligomerization or polymerization reactions, the reactions are promoted by the vesicles through an increase in local concentrations of the reacting monomers and reaction intermediates, and to a co-localization of the monomers and condensing agents or the catalysts. In all cases, the oligomers or polymers obtained remain bound to the vesicles. The vesicles help keeping the reaction products dispersed, preventing product precipitation which may occur due to product aggregation in the absence of vesicles (or other additives). Reaction products which remain associated with the vesicles may lead to changes in the vesicle shape, as illustrated in Fig. 3B. Such changes were observed for the HRPC/H_2O_2-catalyzed polymerization of aniline on the surface of anionic vesicles, either formed from AOT (Fig. 2)[189] or from an equimolar mixture of decanoic acid and sodium dodecylbenzene sulfonate.[191]

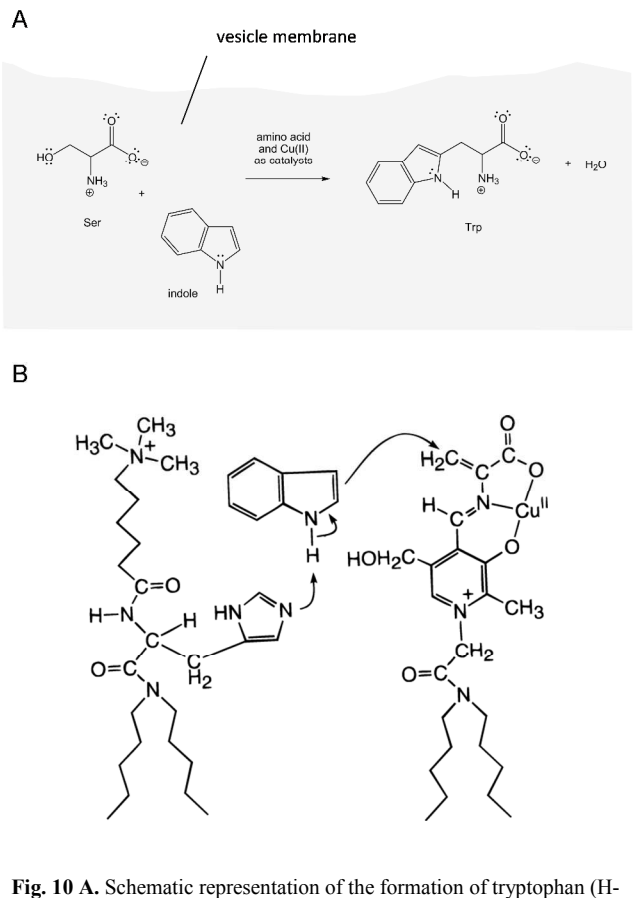


Fig. 10 A. Schematic representation of the formation of tryptophan (H-Trp-OH) from serine (H-Ser-OH) and indole at pH = 5.0 and T = 30 °C in the presence cationic vesicles composed of $PL^{+}2C_{16}$, $N^{+}C_5His2C_{16}$, and Cu(II) ions (see **Fig. 7B)**.[135,180,181] The vesicles used are the same as the ones used for the reaction described in **Fig. 7**. **B.** Illustration of the possible key step of the activation mechanism for the reaction to occur within the vesicle membranes.[135]

In the example shown in Fig. 14, a macromolecular catalyst (HRPC) binds to the outer surface of AOT vesicles, probably mainly through electrostatic interactions. It was also found that this binding leads to a stabilization of the enzyme, *i.e.* the enzyme is more stable than in bulk solution if stored at room temperature without any polymerization reaction taking place.[189,190] The reason for this stabilization is not clear. However, it demonstrate another aspect of the emergent properties vesicle membranes may have, namely a change in the properties of macromolecules through a binding between the vesicles and the macromolecules, just like in the case discussed above and illustrated in Fig. 6, where binding of a short peptide to vesicle membranes may lead to conformational changes so that the peptide becomes a (more efficient) catalyst.

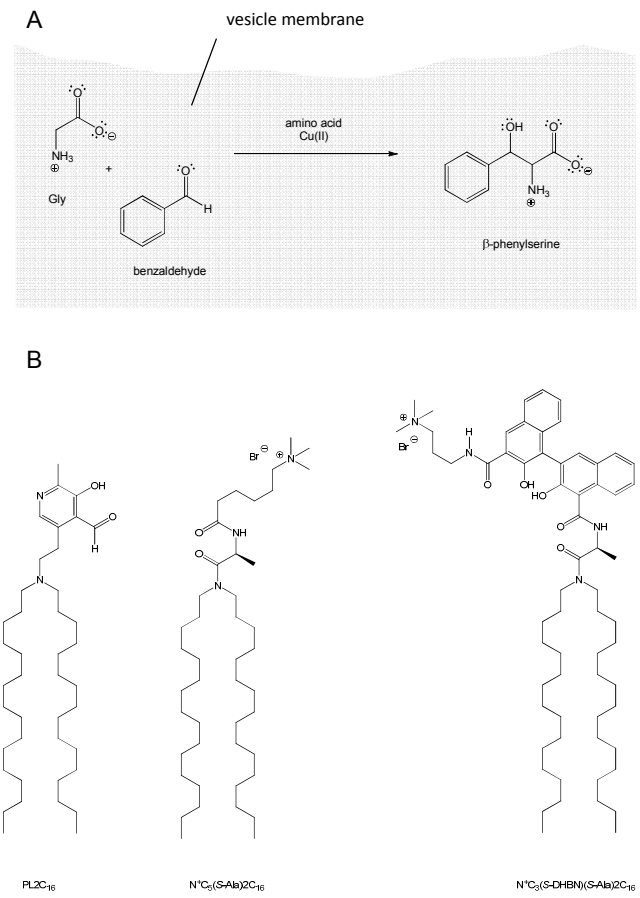


Fig. 11 A. Schematic representation of the synthesis of β-phenylserine from glycine (H-Gly-OH) and benzaldehyde occurring enantioselectively in cationic vesicles at pH = 7.0 and T = 30 °C.[182,183] **B.** The vesicles were composed of $PL2C_{16}$ – which is different from $PL^{+}2C_{16}$ shown in **Fig. 7B**, $N^{+}C_5$(*S*-Ala)$2C_{16}$, $N^{+}C_3$(*S*-DHBN)(*S*-Ala)$2C_{16}$ and Cu(II) ions.[183] DHBN is a binaphthol moiety.

2. 7 Implications of vesicle membrane peptide or protein interactions

The effect lipid vesicles may have on proteins is well known,[192] and it is likely that the modulation of the function of proteins through their interaction with biological membranes is a regulatory mechanism in biological cells. Well investigated is the case of cytochrome c (pI ≈10) which binds to anionic vesicles.[192] If the vesicles contain negatively charged cardiolipin amphiphiles, the interaction of cytochrome c with the vesicle membranes leads to an increase in the peroxidase activity of this heme protein (through a change of the redox potential caused by conformational changes),[193, 194] and to an increase in the permeability of the vesicle membranes through the formation of pores (Fig. 15).[195] In experiments with giant unilamellar vesicles (GUVs with sizes between about 10 and 15 μm) formed from a mixture of DOPC and bovine heart cardiolipin (10-20 mol%) at pH = 7.4 it was shown that the pores formed allowed the transport of molecules from the exovesicular medium into the endovesicular space for molecules with molar masses of up to about 10,000 g/mol.[195] Therefore, the specific interaction between

the vesicle membrane and cytochrome c leads to changes of the protein *as well as* to changes of the vesicle membrane properties. If large unilamellar vesicles (LUVs with a diameter of about 100 nm) from POPC were used, guanidinium hydrochloride (GuHCl)-denatured cytochrome c was shown to lead to vesicle fusions due to interactions between the hydrophobic parts of the protein, which become exposed after treatment with GuHCl, and the vesicle membranes.[196] Additional examples in which the effect of vesicle membranes on the properties of proteins was demonstrated are listed in Table 3.[197-201] Many more examples can be found in the literature.

The effect of lipid assemblies as modulators of proteins is a topic of research on its own,[195] since it is evident that not only the interactions between amphiphiles of biomembranes and transmembrane proteins are relevant for the functioning of these proteins, but there are also interactions between membranes and certain water soluble or peripheral proteins which can be of great importance for the functioning of biological cells. Given oligopeptides or proteins (*e.g.* antimicrobial pore-forming peptides)[202, 203] are only biologically active upon their interaction with biological membranes. On the other hand protein-membrane interactions may also be responsible for the development of certain diseases (Fig. 16).[201]

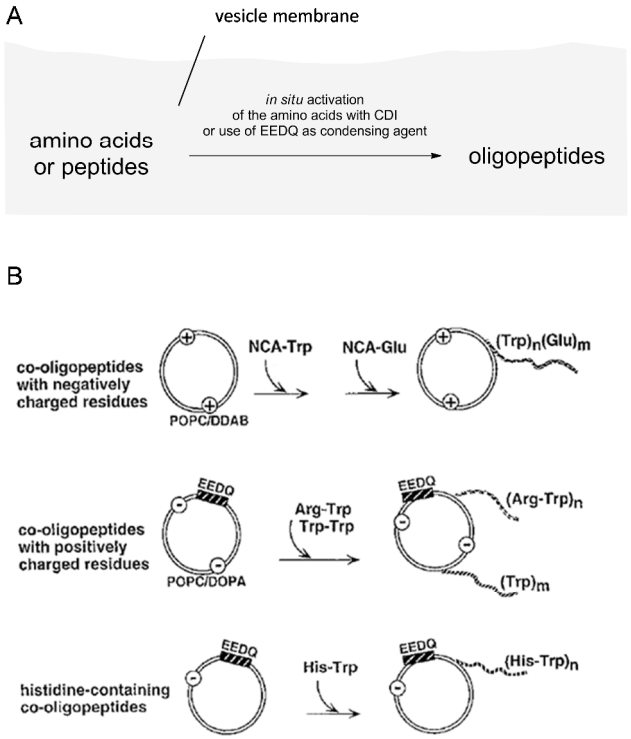


Fig. 12 A. Schematic representation of the vesicle-assisted oligomerization of amino acids.[184, 185] **B.** Activated amino acids (*N*-carboxyanhydrides, NCAs) which bind to the vesicles can be used in a sequential reaction, *e.g.* NCA-Trp first and then NCA-Glu - obtained from H-Trp-OH and H-Glu-OH and CDI (*N,N'*-carbonyldiimidazole) - with cationic vesicles formed from POPC and DDAB at pH = 7.5 and T = 25 °C);[185] or peptides which are coupled with the lipophilic condensing agent EEDQ (*N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline) at pH = 6.4 and T = 25 °C with anionic vesicles formed from POPC and POPA.[185] Drawing from Blocher *et al.*[185]

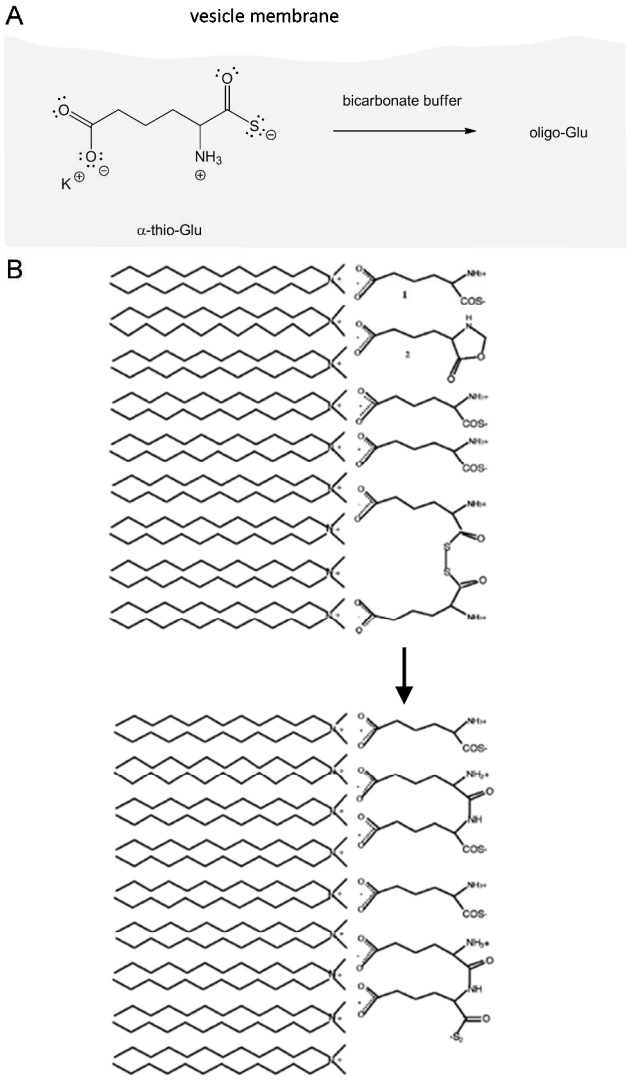


Fig. 13 A. Schematic representation of the formation of oligo-glutamic acids as obtained from the potassium salt of α-thioglutamate in bicarbonate buffer in the presence of cationic vesicles formed from di-*n*-dodecyldimethylammonium bromide.[186] **B**. Illustration of the possible reaction steps on the surface of the vesicle membrane. Through a reaction between α-thioglutamate with bicarbonate in solution, *N*-carboxyanhydrides and disulfide linkages are formed. After reaction, dipeptides are formed which then bind to the vesicle membrane together with α-thioglutamate. The close proximity on the vesicle surface promotes further reactions to yield oligopeptides. Drawing from Zepik *et al.*[186]

The possibility of co-localizing proteins (enzymes), metal-binding ligands and metal ions within fluid vesicle membranes was the basis for the elaboration of sophisticated vesicular signaling systems by Kikuchi *et al.* (Fig. 17). [204-206] The vesicle system shown in Fig. 17 is composed of a cationic amphiphile ($N^+C_5(Gly)2C_{16}$) and of a steroidal amine, prepared at pH = 7.0. After addition of 1-hydroxy-2-naphtylaldehyde, the imine is formed which can complex Cu(II) ions, as shown in Fig. 17A(b). Binding of the metal ions leads to a rearrangement of the amphiphiles within the membrane. Added NADH-dependent pig

heart lactate dehydrogenase (LDH) binds to the vesicle membranes and the activity can be measured with pyruvate as substrate which is oxidized to L-lactate, as measured by the reduction of NADH to NAD^+. The activity of LDH is inhibited by Cu(II) ions. In the entire system the activity of LDH is controlled by the presence of the metal chelating ligand, *i.e.* inactive LDH can be activated though the addition of 1-hydroxy-2-naphtylaldehyde as activator molecule.[204] The system shown in Fig. 17 was also modified by using a different activator molecule, and $N^+C_5(Ala)2C_{16}$ as amphiphile with DMPE (1,2-dimyristoyl-*sn*-glycero-3-phosphoetahnolamine) as amine,[205] or with DPPC (or DMPC or DSPC) and DMPE.[206] In this latter case, the effect of the fluidity of the vesicle membrane was shown to have a significant influence on the performance of the signaling system, the LDH activity was more sensitive in the s_o-state of the membrane.[206] A further variation of the system was the inclusion in vesicle membranes formed from $N^+C_5(Ala)2C_{16}$ of a light-sensitive amphiphiphile (with an azo-benzene moiety) as Cu(II) binding molecule, whereby Cu(II)-binding was observed in the *cis*-configuration only (obtained through UV light irradiation) and not in the *trans*-configuration (after visible light irradiation).[207] In this way, the activity of LDH bound to the vesicles in the presence of Cu(II) ions could be controlled by light.[207] Furthermore, in related experiments, the activity of LDH adsorbed onto vesicle membranes could be controlled *via* a oligonucleotide hybridization occurring on the surface of the membranes.[208] A vesicle-bound oligonucleotide lipid (cholesterol used as membrane anchor) was integrated into cationic vesicles formed from $N^+C_5(Ala)2C_{16}$ with an average size of 120-124 nm at pH = 7.0. Addition of a complementary oligonucleotide led to a hybridization which in turn led to an increased binding of Cu(II) ions. In this way Cu(II)-inactivated surface bound LDH could be reactivated.[208] For all these experiments with LDH it is worth noting that experiments with micelle-forming CTAB (*n*-hexadecyltrimethylammonium bromide) failed since this surfactant denatures the enzyme.[207, 208]

2. 8 Specific interactions between vesicle membranes and oligo- or polynucleotides

These last examples illustrate how the function of an enzyme can be modulated within a vesicle membrane through a co-localization of various specifically designed molecules *via* photochemical or chemical signals, the latter including oligonucleotides. In Table 4, other selected examples are listed which are based on membrane-oligonucleotide or membrane-nucleic acid interactions.[209-212] In Fig. 18, results from a study with GUVs are summarized. T4 DNA was fluorescently labeled and then added to cationic vesicles formed from a mixture of DOPC, cholesterol, DOPE or DPPE, and a small amount of rhodamin-DOPE (for visualizing micro-domain formation within the membrane since this fluorescent lipid preferentially resides in the l_d-phase, see also Fig. 1G). Since the surface charge density, which depends on the phase state, determines the binding of the externally added DNA to the vesicle membrane, the DNA binding was controlled by the membrane composition. Phase-separated vesicle membranes (l_o-state containing DPPE) adsorb DNA with an elongated conformation (Fig. 18B), while there is only weak interaction between vesicle membranes in the l_d-state and the DNA in its coiled conformation (Fig. 18A).

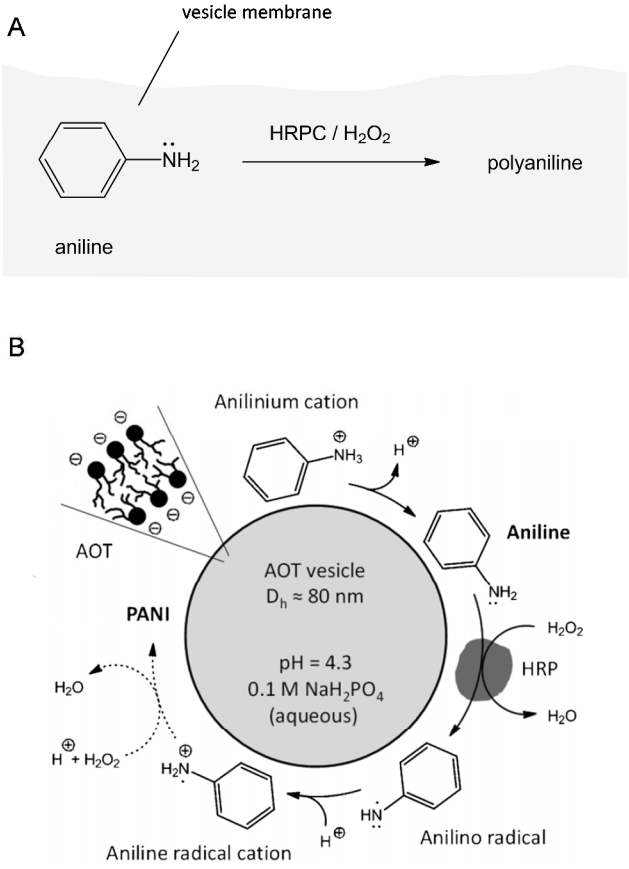


Fig. 14 A. Schematic representation of the polymerization of aniline to polyaniline with the enzyme horseradish peroxidase isoenzyme C (HRPC) and H_2O_2 as catalyst and oxidant. **B.** Illustration of the reaction occuring on the surface of anionic vesicles formed from AOT at pH = 4.3 and T = 25 °C. The polymerization reaction occurs localized on the surface of the vesicles through a binding of the catalyst (HRPC) and the monomers (aniline and the anilinium cation ion). After oxidation of aniline to the anilino radical and subsequent protonation to the anilino radical cation, the polymerization occurs on the vesicle surface, whereby the vesicles influence the regioselectivity of the coupling reactions.[189,190] Drawing from Junker *et al.*[190]

2. 9 Possible applications of vesicle membranes as reaction promoters and regulators

Some of the experimental observations mentioned above are important and of interest to those who like to better understand some of the fundamental physico-chemical aspects of processes which occur within biomembranes or on the surfaces of biomembranes since they are essential for all forms of life. Furthermore, some of the results obtained so far contribute to the development of biomembrane-mimicking systems and biosensor and signaling devices.[204–206] Since vesicle formation is even possible from polymeric amphiphiles, possible applications may also be found in future in material science. The vesicle surface area in a system containing dispersed vesicles can be very large. Therefore, vesicle membrane-promoted or –regulated reactions can occur very efficiently which is useful for possible

applications. The calculated external surface area of a suspension of unilamellar POPC vesicles (1 mM POPC) with a vesicle diameter of 0.1 μm (assuming a hydrophilic head group area[78] of 0.72 nm^2 and a calculated aggregation number of about 85 000) is 4.5 $m^2 \cdot mL^{-1}$, with a calculated concentration of vesicles of $1.18 \cdot 10^{-8}$ M.

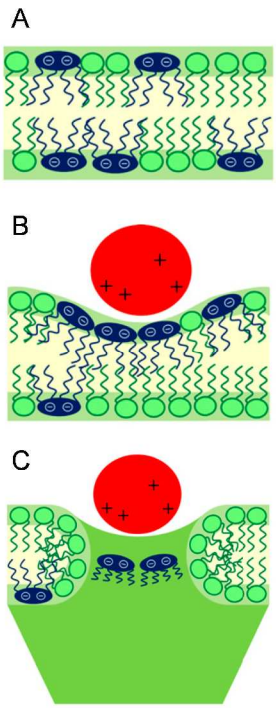


Fig. 15 Schematic representation of the proposed interaction of cytochrome c with a vesicle bilayer composed of DOPC (amphiphile with green head group) and anionic cardiolipin (amphiphile with blue head group).[195] **A.** The cardiolipin molecules are constrained to lie flat in the bilayer with DOPC. **B.** Cytochrome c (red) interacts with the vesicle membrane and leads to a clustering of the cardiolipin molecules and reduces head group repulsions, leading to the formation of negative curvature structures. **C.** In a next step, an inverted structure may form within the membrane, *e.g.* an inverted hexagonal state or a "small-diameter toroidal lipid pore" (shown),[195] which makes the vesicle membrane permeable for molecules with molar masses up to about 10,000 g/mol. Image adapted from Bergstrom *et al.*[195]

What are some of the great challenges in this field of research? They are related to the key feature (IV) of vesicle systems mentioned above (section 1.4., Fig. 3B). In the majority of the amphiphile assemblies which have been studied so far, the amphiphiles forming the assemblies do not undergo chemical transformations to a significant extent during the period of investigation with the exception of protonation or deprotonation reactions in case the amphiphiles are weak acids or bases. This limitation is fully understood if one is, for example, interested in determining the size of an assembly "object" as it is obtained through the particular methodology applied. It, however, eliminates completely potentially interesting dynamic aspects of the entire system as they are characteristic for all forms of life, for example the formation and transformation of vesicle membrane-forming molecules leading to vesicle compartment growth and division processes. One of the great challenges is to combine chemical reactions taking place within and on the surface of vesicles with concomitant changes of vesicle shape and vesicle number, as this is a physico-chemical characteristic of all forms of life (cell-division).[45, 57]

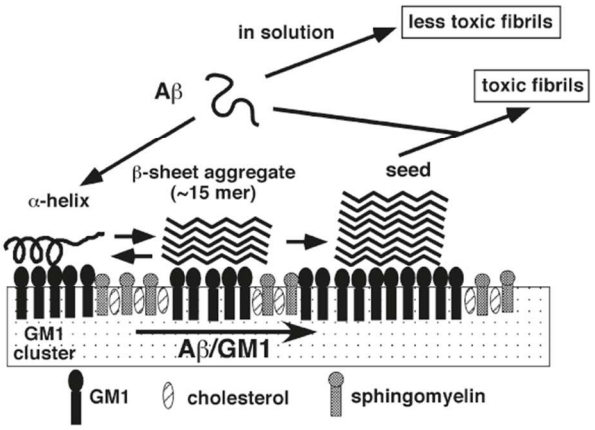


Fig. 16 Schematic representation of the proposed interaction of amyolid-*β*-protein (A*β*) with membranes containing domains of GM1.[201] A*β* specifically binds to a GM1 cluster, leading to a change in the conformation of A*β* from random coil to an α-helix-rich conformation. Depending on the ratio of A*β* to GM1, peptides rich in α-helix conformations coexist with aggregated β-sheets which may turn into toxic amyloid fibrils which may be relevant for the development of Alzheimer's disease, a severe neurodegenerative disorder.[201] Image from Ikeda *et al.*[201]

3 Possible relevance of vesicle membrane-associated reactions for the emergence of functionalized protocells

As discussed above (section 1.2.), the formation of vesicular structures probably was an important event in the history of life on Earth. Although nobody knows how the first living systems emerged from the non-living form of matter, one assumes that first various chemically simple molecules formed on the early Earth or they were delivered from the interstellar space *via* carbonaceous meteorites to the Earth.[213] Particularly challenging is to understand those steps in which a mixture of prebiotic molecules in an environment which was rich in water and inorganic materials eventually led to the *first systems one would consider to be the first forms of life*. This remains a mystery, very difficult to understand and to model since all forms of life are so complex. Part of the research in the field of "systems chemistry"[34, 214] deals with these issues and tries to understand through theoretical considerations and experimental studies how interactions in mixtures of different types of molecules and between molecules and inorganic or organic interfaces can lead to the formation of molecular assemblies and more complex functional molecules, *i.e.* how new properties can emerge on the basis of non-biological physical and chemical processes. Fig. 19 is a schematic representation of a hypothetical protocell.[215] Obviously, this type of protocell already is a rather sophisticated system which is composed of complex molecules, including different types of macromolecules, and the question is, how this molecular and organizational complexity could have arisen abiotically. Assemblies of amphiphilic molecules,[32, 216

particularly vesicle membranes, may have contributed to the synthesis and modification of chemical species through the emergent properties vesicle membranes provide when they form from amphiphiles, as outlined in this paper.

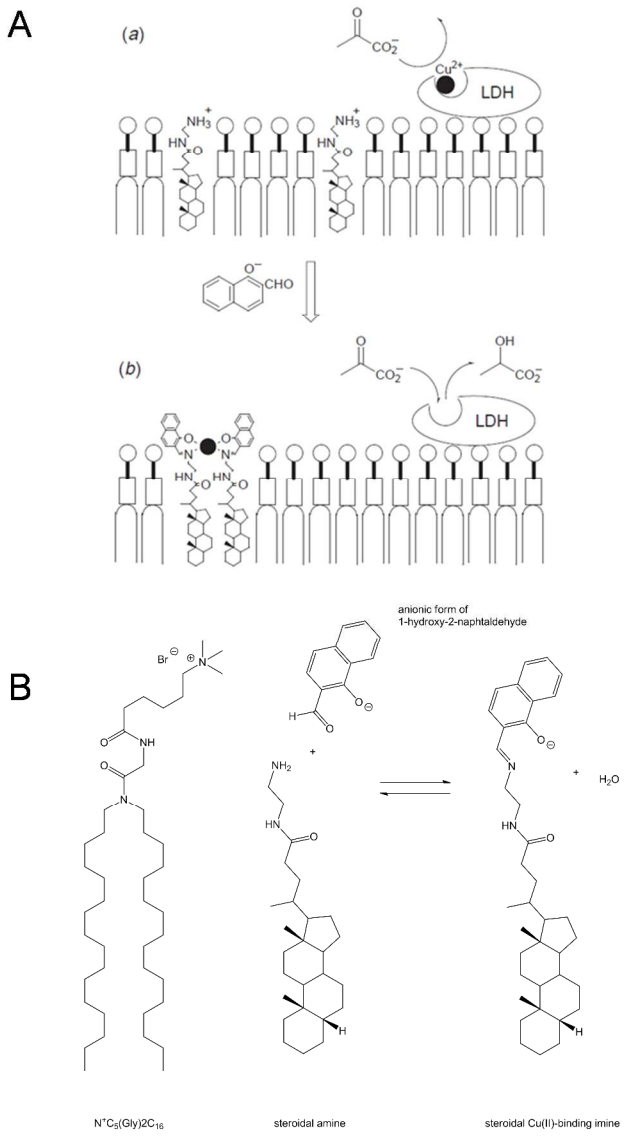


Fig. 17 A. Schematic representation of vesicle system which contains bound LDH (pig heart lactate dehydrogenase), a membrane associated steroidal amine, Cu(II) ions, the anionic form of 1-hydroxy-2-naphthaldehyde, and pyruvate as substrate for LDH (not shown is NADH which is the cofactor of LDH). (a) Situation without added 1-hydroxy-2-naphthaldehyde, indcating that the binding of Cu(II) to LDH leads to an inactivation of the enzyme. (b) Situation after addition of 1-hydroxy-2-naphthaldehyde which reacts to form a Cu(II)-binding imine, leading to a rearrangement of the molecules within the membrane and to a removal of Cu(II) from the active site of the enzyme, and therefore to an activation of LDH, catalyzing the oxidation of pyruvate to L-lactate under simultaneous reduction of NADH to NAD^+ (not shown). The vesicles used had a diameter of about 120 nm. Drawing adopted from Kikuchi *et al.*[204] **B.** Chemical structures of the cationic amphiphile used, of the steroidal amine, and of the formation of the imine.

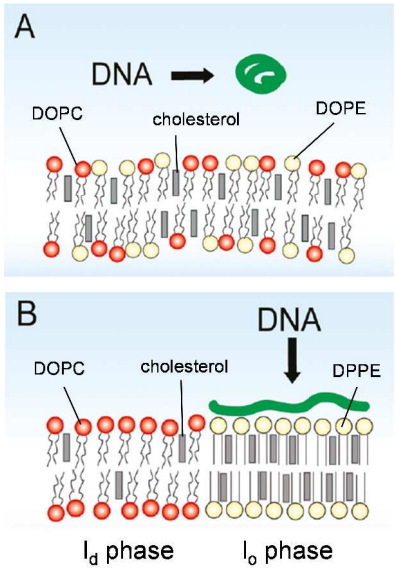


Fig. 18 Schematic representation of the interaction of giant T4 DNA with giant vesicle bilayers composed of DOPC, cholesterol, and DOPE (**A**), or DOPC, cholesterol, and DPPE (**B**). In the fluid l_d-state, the coiled DNA does not adsorb, while adsorption with an elongated DNA conformation occurs onto bilayer domains in their l_o-state, which has a higher charge density that the l_d-state. Drawing adopted from Kato *et al.*,[212] and slightly modified.

Prebiotic membranes may have promoted and regulated the synthesis of complex molecules through a co-localization of reacting molecules at or within vesicle membranes. Since vesicle membranes are soft or semi-solid interfaces, vesicle shape changes may have occurred as a result of reactions taking place in the area of the membranes, as illustrated in Fig. 3B. Therefore, in addition to all those aspects related to the encapsulation of solutes inside vesicles, emerging properties associated to the vesicle membranes can be relevant within the frame of a scenario originally proposed by Blobel,[123] see Fig. 20 and more recently discussed by Griffiths.[125] The outer surface of prebiotic vesicular compartments may have served as reaction promoter and regulator. In this way there was no need for the permeability of the reacting species from the exovesicular medium into the endovesicular volume. The vesicle membrane could have hosted catalysts, in a similar way like in the different examples mentioned above in which, however, mainly contemporary − and not potentially prebiotic − molecules were used. The vesicle-bound reaction products may have led to changes in the permeability of protocell membranes, or they could have become internalized through shape changes and membrane fusion processes (Fig. 3B and Fig. 20). Although all this is pure speculation, it is certainly worth considering vesicle membrane-bound reactions[184-188] when dealing with prebiotic chemistry questions, as alternative to reactions which may have occurred on the surface of minerals.[188, 217, 218]

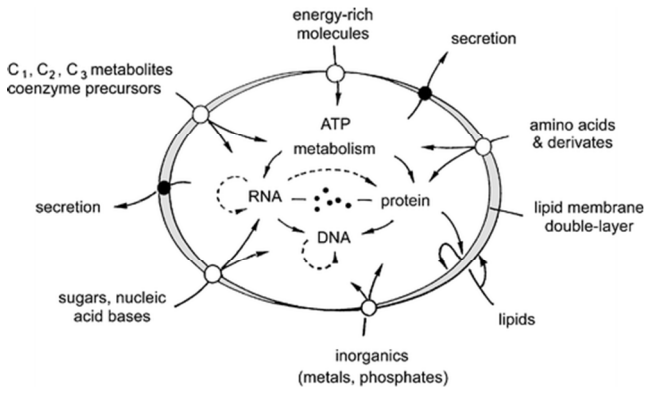


Fig. 19 Schematic representation of a highly sophisticated protocell, indicating essential material transformations (solid arrows) and information transfer (dashed arrows) from an exterior medium and inside a protocell. The dots in the center of the protocell represent RNA-protein particles (ribosomes). The protocell shell is a lipidic membrane which must include hydrophobic receptor or channel proteins for the controlled uptake of nutrients (open circles) and leaky patches for the secretion of waste (closed circles). Drawing from Follmann and Brownson.[215]

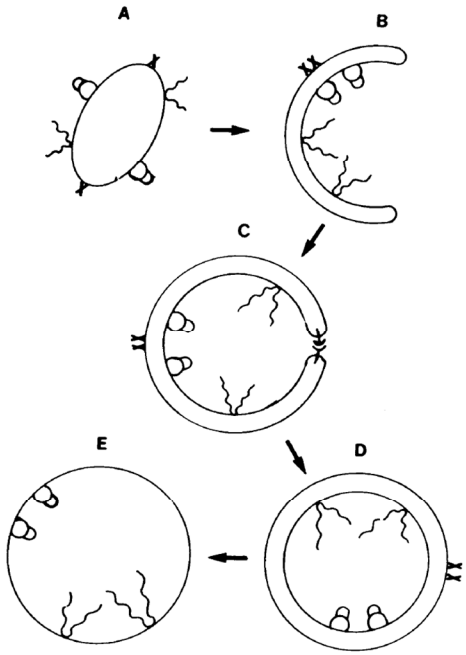


Fig. 20 Schematic illustration of various theoretical stages of the possible precellular evolution on the surface of vesicles which may have led to the formation of the first cells, as proposed by Blobel.[123] In this scenario, it is assumed that complex macromolecules were already present. (A): Vesicles containing integral membrane proteins (nor shown) are able to bind on the outer vesicle membrane various macromolecules (X) and macromolecular complexes, among them chromatin and ribosomes. (B) Non-random distribution of the boudn components on the vesicle surface and beginning of invagination. (C) Formation of a "gastruloid" vesicle, perhaps able to open and to close *via* protein-protein interactions of membrane embedded proteins at its orifice. (D) Fusion at the orifice, resulting in a primordial cell delimited by two membranes. (E) Loss of the outer membrane. Drawing and legend text (with small modifications) adopted from Blobel.[123]

Table 1 Examples of the influence of chemical reactions by vesicle membranes

Amphiphiles (vesicle type and size)[a]	Experimental observations, interpretation of the observations made, significance of the observations	References
DLPC, DMPC, DPPC, DSPC (LUVs, ≈100 nm)	A lipophilic porphyrin derivative is integrated into the vesicle membranes and found to catalyze the dismutation of superoxide radial anions ($O_2^{\bullet-}$) into H_2O_2 and the decomposition of H_2O_2 into H_2O and O_2, the efficiency being dependent on the fluidity of the vesicle membrane. Highest activity at 25 °C and pH ≈7 is observed with DMPC vesicles (T_m = 24 °C). The vesicle membrane protects the interior from external oxidative stress.	Umakoshi *et al.*[177]
DLPC, DMPC, DPPC, POPC, DOPC (LUVs, ≈100 nm)	The decomposition of H_2O_2 to H_2O and O_2 is catalyzed by the vesicles, the effect at 25 °C and pH = 7.4 being largest with DMPC vesicles (T_m = 24 °C). The experiments show that the coexistence of l_d and s_o domains in the vesicle membrane is most effective, indicating that the reactivity is regulated by the physico-chemical state of the vesicle membrane.	Yoshimoto et al.[178]
Egg PC (LUVs, ≈200 nm)	Investigations of reversible thioester exchange reactions which occur in competition with the hydrolysis of the thioesters at pH = 7.0 or 8.0 indicate a shift of the product formation towards the formation of vesicle membrane-bound products, leading to a shift from macrocyclic towards linear products. First system in which the outcome of dynamic combinatorial chemistry reactions is regulated by vesicle membranes.	Manseld *et al.*[229]

[a] POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; DLPC, 1,2-dilauroyl-*sn*-glycero-3-phosphocholine; DMPC, 1,2-myristoyl-*sn*-glycero-3-phosphocholine; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; DSPC, 1,2-stearoyl-*sn*-glycero-3-phosphocholine; DOPC, 1,2-dioleoyl-*sn*-glycero-3-phosphocholine; egg PC; mixture of phosphatidylcholines isolated from chicken egg yolk; LUVs, large unilamellar vesicles.

Table 2 Oligomerization and polymerization reactions occurring on the surface of vesicle membranes

Amphiphiles (vesicle type and size)[a]	Experimental observations, interpretation of the observations made, significance of the observations	References
POPC (LUVs, ≈100 nm)	The oligomerization of *N*-carboxyanhydride (NCA)-L-Trp is influenced by the presence of the vesicles, whereby longer chains are obtained in the presence of the vesicles as compared to a reaction carried out without vesicles, >20 mer *vs* 7 mer. The vesicle membrane provides a medium for solubilizing water insoluble oligopeptides.	Blocher *et al.*[184]
POPC/DDAB, POPC/DOPA (LUVs, ≈100 nm)	The oligomerization of charged NCA-amino acids - or of charged dipeptides with the hydrophobic condensing agent EEDQ - in the presence of oppositely charged vesicles leads to the formation of oligopeptides (from Trp, Glu, Arg, and His). Accumulation of the condensing agent, the activated amino acids, the dipeptides and the intermediate reaction products on the surface of the vesicles appears responsible for the observed oligopeptide formation. Fig. 12	Blocher *et al.*[185]
DDAB (<800 nm)	Upon addition of α-thioglutamate in bicarbonate buffer, the formation of oligoglutamates is observed (up to 13-14 mer) with concomitant vesicle aggregation and fusion, whereas in the absence of amphiphiles, only up to 4 mers can be detected. It is likely that competing reactions (hydrolysis and formation of cyclic products) are minimized if the reaction occurs on the surface of the vesicles. The vesicle membranes cause a change in the reaction pathways towards the formation of oligomeric products. **Fig. 13**	Zepik *et al.*[186]
POPC, POPA (<1 μm)	Oligomerization of AMP to RNA-like polymers can be achieved if AMP is added to the vesicles, followed by dehydration-rehydration cycles. The organizing effect of the vesicle system promotes the oligomerization reaction.	Rajamani *et al.*[187]
AOT (LUVs, ≈80 nm)	Polymerization of aniline (pK_a (anilinium) = 4.6) occurs on the surface of the anionic vesicles with the help of the isoenzyme C of horseradish peroxidase (HRPC, pI = 8.8) as catalyst and H_2O_2 as oxidant. Under optimal reaction conditions (pH = 4.3, T = 25 °C), polyaniline (PANI) is obtained as emeraldine salt (PANI-ES), the conductive form of this polymer. HRPC/H_2O_2 oxidizes aniline monomers which then react with other aniline monomers to form long polymeric chains. The actual polymerization step is not under direct control of the enzyme, but rather assisted by the vesicle membranes, whereby the anionic amphiphiles forming the vesicles are also the counter ions (dopants) of PANI-ES.	Guo *et al.*[189]; Junker *et al.*[190]

[a] POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine, DDAB, di-*n*-dodecyldimethylammonium bromide; DOPA, 1,2-dioleoyl-*sn*-glycero-3-phosphate; POPA, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphate; AOT (sodium bis(2-ethylhexyl)sulfosuccinate); LUVs, large unilamellar vesicles.

Table 3 Examples of vesicle membranes as modulators of the properties of proteins (activity of enzymes)

Amphiphiles (vesicle type and size)[a]	Experimental observations, interpretation of the observations made, significance of the observations	References
POPC (LUVs, ≈100 nm)	The refolding of guanidinium hydrochloride (GuHCl)-denatured carbonic anhydrase is influenced by the vesicles. Possible binding of the molten globule state of the enzyme to the vesicle membranes accelerates the refolding process through a change in the reaction pathway. The vesicle membrane acts as protein folding promoter.	Kuboi *et al.*[197]
DOPC; DMPC/DOPC; DOPC/DOPE (GUVs, ≈2 μm)	The activity of CTP:phosphocholine cytidyltransferase (CCT) is modulated through interactions with the vesicle membranes, whereby high enzyme activity is observed with vesicle membranes that have a high stored curvature elastic energy (no phase separation). This "torque tension" is reduced after binding of the enzyme, promoting efficient binding which then results in an increased enzyme activity. Changes in the physico-chemical properties of vesicle membranes by varying the membrane-forming amphiphiles can regulate the activity of vesicle membrane-bound enzymes.	Attard *et al.*[198]
POPC (LUVs, ≈100 nm)	Inactive fragments of H_2O_2-treated superoxide dismutase (SOD) regain enzymatic activity in presence of the vesicles due to binding of the fragments to the vesicle membrane with most likely induces changes in the conformation of the fragments from an inactive to an active state.	Tuan *et al.*[199]
DOPC; DOPC/oleic acid (GUVs, ≈2 μm)	The activity of 6-phosphofructo-1-kinase is enhanced in the presence of the vesicles. Analysis of the kinetic data indicates that the interaction of the enzyme with the vesicle membrane leads to an extraction of the lipids from the membrane which results in conformational changes of the enzyme. The vesicle membrane acts as enzyme activator.	Tsaloglou *et al.*[200]
GM1/cholesterol/SM (SUVs, <100 nm)	Amyloid-*β*-protein (A*β*) monomer interacts with the vesicles. Depending on the composition of the vesicles, the interaction may lead to the formation of protein assemblies (β-sheets) which may further convert into larger aggregates ("seeds") and finally into toxic fibrils. Seed formation is observed in vesicle membrane domains which are rich in GM1. Fig. 16.	Ikeda *et al.*[201]

[a] POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; DOPC, 1,2-dioleoyl-*sn*-glycero-3-phosphocholine; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; DOPE, 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine; GM1, monosialoganglioside from bovine brain; SM, *N*-acyl-D-sphingosine-1-phosphocholine (sphingomyeline) from bovine brain; SUVs, small (sonicated) unilamellar vesicles; LUVs, large unilamellar vesicles; GUVs, giant unilamellar vesicles.

Table 4 Examples of the effect of the interaction between vesicle membranes and oligonucleotides or nucleic acids

Amphiphiles (vesicle type and size)[a]	Experimental observations, interpretation of the observations made, significance of the observations	References
POPC/cholesterol (LUVs, ≈100 nm)	The presence of the vesicles leads to an enhanced protein expression in a cell-free gene expression system from *E. coli*, the effect being dependent on the fluidity of the vesicle membrane.	Bui *et al.*[209]
POPC/cholesterol; POPC/DOTAP	Interaction of tRNA and mRNA with the vesicle membranes leads to conformational changes, the extent being dependent on the vesicle membrane composition. For mRNA, the interaction with the vesicle membrane leads to a slight increase or to a decrease in protein expression (GFP) if a *E.coli* cell-free translation system is used.	Suga *et al.*[210]
DOPC/DOTAP; DOPC/DC-Ch (LUVs, ≈100 nm)	The conformation of mRNA and its translational activity in a cell-free *E. coli* translation system for GFP are altered in the presence of the vesicles, the effect being dependent on the overall positive charge and the phase state of the amphiphiles. GFP expression is inhibited by the vesicles, whereby the extent of inhibition is most pronounced with highly charged vesicles in the liquid disordered state (l_d). The vesicle membranes act as regulators of protein expressions.	Suga *et al.*[211]
DOPC/DOPE/cholesterol; DOPC/DPPE/cholesterol (GUVs, ≈15-30 μm)	DNA selectively adsorbs on liquid ordered (l_o) domains of phase-separated vesicle membranes. This phase-selective binding can be explained by a higher surface charge density in the l_o phase as compared to the l_d phase. DNA adsorption leads to a change of the conformation from a compact DNA conformation to an elongated conformation. Fig. 18	Kato *et al.*[212]
DMPC; DOPC/SM/cholesterol (LUVs, ≈100 nm; GVs, 2-10 μm)	RNA preferentially binds to the solid ordered (s_o) and liquid ordered (l_o) phase and not to the liquid disordered (l_d) phase, whereby binding to the l_o phase is RNA structure dependent, while this is not the case for the s_o phase. RNA structure and membrane order modulates RNA-membrane interactions.	Janas *et al.*[230]

[a] DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; DOTAP, 1,2-dioleoyl-3-trimethylammonium-propane chloride; DC-Ch, 3*β*-[*N*-(*N*′,*N*′-dimethylaminoethane)-carbamoyl]cholesterol hydrochloride; DPOC, 1,2-dioleoyl-sn-glycero-3-phosphocholine; DOPE, 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine; DPPE, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; SM, *N*-stearoyl-D-erythro-sphingosylphosphorylcholine (stearoyl sphingomyelin); LUV: large unilamellar vesicle; GV: giant vesicle; GUV: giant unilamellar vesicle

4 Conclusions and outlook

Among the different properties which emerge from the formation of artificial vesicles, we limit ourselves in this paper to a few selected examples in which vesicles act as promoter or regulator of chemical reactions through a localization of the reactions in the region of the vesicle membrane. Conceptually, this is in analogy to reactions taking place at membranous interfaces in biological systems. Possible fields of applications of such "vesicle-assisted" reactions are for example in bioanalytics, biotechnology and in synthetic chemistry.

One of the great challenges in using vesicles as compartmentalized reaction systems is the investigation of a set of interdependent reactions taking place in the system, possibly partially localized in the region of the vesicle membrane, within the vesicles and in the bulk medium with a controlled exchange of solutes between the bulk medium and the vesicle's interior. Such network of reactions may lead to changes in the vesicle system in a way that the properties of the vesicles are changed during the reaction, i.e. the vesicle membrane permeability is altered selectively, and that simultaneously the vesicle concentration is increased through controlled vesicle growth and division processes. All of this is very difficult to achieve experimentally, but first encouraging results from related studies have been obtained over the last years.[45, 93, 101, 219-221] This type of research work is mainly driven by two main questions, namely (i) is it possible to synthesize minimal cell-like systems, and (ii) is it

possible to understand the origin of the first cells? All those interested in these two questions have to deal with the chemistry and physics of complex compartmentalized molecular systems which are able to undergo morphological changes; whereby one of the difficulties is not only to design and to mathematically formulate a system of interest, but also to be able to analyze quantitatively chemical and physical transformations occurring in the system. In this perspective, computational modeling can help researches involved in this field - both by investigating engineered vesicles before their experimental implementation for suggesting a possible optimization of the preparation procedures, or by describing scenarios which are difficult to monitor experimentally. Dealing with complex compartmentalized systems undergoing morphological transformations is one of the fascinating and stimulating challenges of systems chemistry as scientific bottom-up counterpart discipline to systems biology.[34,214]

Acknowledgement

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Notes and references

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‡ Dedicated to Prof. Pier Luigi Luisi and Prof. David W. Deamer, two of the pioneers in protocell research.

1. The term "amphiphile" specifies a particular class of molecules. They bear in their chemical structure "hydrophilic" parts, which have a high solubility in water but low solubility in oil, and "lipophilic" parts with a high solubility in oil but low solubility in water. Here, "water" does not strictly mean pure "H_2O" but stands for any type of aqueous solution; and the term "oil" refers to a lipidic medium which is not miscible with water, *e.g.* hexane. An extension of the general concept of amphiphilicity is the inclusion of molecules which have "fluorophilic" groups which are highly soluble in fluorocarbons, *e.g.* perfluorohexane, but have low solubility in water as well as in "oil".
2. Note that vesicles can also form from amphiphiles in non-aqueous solvents. This is, however, not considered here.
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