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From Self-Assembled Vesicles to Protocells

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Self-assembled vesicles are essential components of primitive cells. We review the importance of vesicles during the origins of life, fundamental thermodynamics and kinetics of self-assembly, and experimental models of simple vesicles, focusing on prebiotically plausible fatty acids and their derivatives. We review recent work on interactions of simple vesicles with RNA and other studies of the transition from vesicles to protocells. Finally we discuss current challenges in understanding the biophysics of protocells, as well as conceptual questions in information transmission and self-replication.

For synthetic biologists, a useful operational definition of life is "a self-sustaining chemical system capable of Darwinian evolution," which was adopted by the Exobiology program of NASA (Joyce 1994). In the quest to build a simple living system, much recent interest has focused on encapsulating a genetic or metabolic system inside membrane vesicles (Deamer and Dworkin 2005; Luisi et al. 1999; Morowitz et al. 1988; Ourisson and Nakatani 1994; Szostak et al. 2001). Vesicles are supramolecular aggregates containing an aqueous interior that is separated from the bulk solution by one or more bilayers of amphiphiles.

Why use vesicles? Although they are not strictly required in this definition of life, there are two major reasons why vesicle membranes are thought to be important. The first is that the membrane forms a semipermeable barrier that permits small molecules to pass into the cellular space and traps modified (e.g., phosphorylated or polymerized) products. The second reason is evolutionary: The membrane separates different genomes from one another and reduces the problem of inactive parasites (Szathmary and Demeter 1987; Szostak et al. 2001). During the origin of life, physical grouping is a plausible way for replicator enzymes (replicases) to interact nonrandomly. For example, replicases encapsulated in growing and dividing membrane vesicles would tend to be trapped with sequences related to their own sequence, and thus would preferentially copy those sequences. Because the vesicles separate different genomes from each other, poor replicases would not have access to active replicases, whereas mutants with improved replicase activity would benefit directly themselves, as their descendants remain in the same vesicle and copy each other. An occasional parasitic sequence would

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be separated from most of the active polymerases during vesicle division and could not poison the entire system (the "stochastic corrector" model) (Smith and Szathmary 1995; Szathmary and Demeter 1987). Thus, a higher level of population organization, the cell, greatly facilitates the evolution of more efficient replicases (Cavalier-Smith 2001; Koch 1984; Matsuura et al. 2002; Szathmary and Demeter 1987; Szostak et al. 2001).

Membrane vesicles are not the only way to segregate different genomes. The attachment of molecules onto surfaces also creates a heterogeneous distribution of interactions based on spatial proximity, a scenario that has been investigated theoretically using cellular automata models (Szabo et al. 2002). Although they may not have been the initial means of achieving genomic segregation during the origin of life, membranes are the dominant means of separating cells today. Membranes presumably assumed this function very long ago, at least three to four billion years ago, at some time before the diversification from the last common ancestor.

Vesicle morphologies and topologies can cover a rich and diverse landscape (Fig. 1, top), although experimentalists tend to prefer unilamellar vesicles because data can be more easily interpreted in this context, and because these vesicles resemble contemporary cells, which

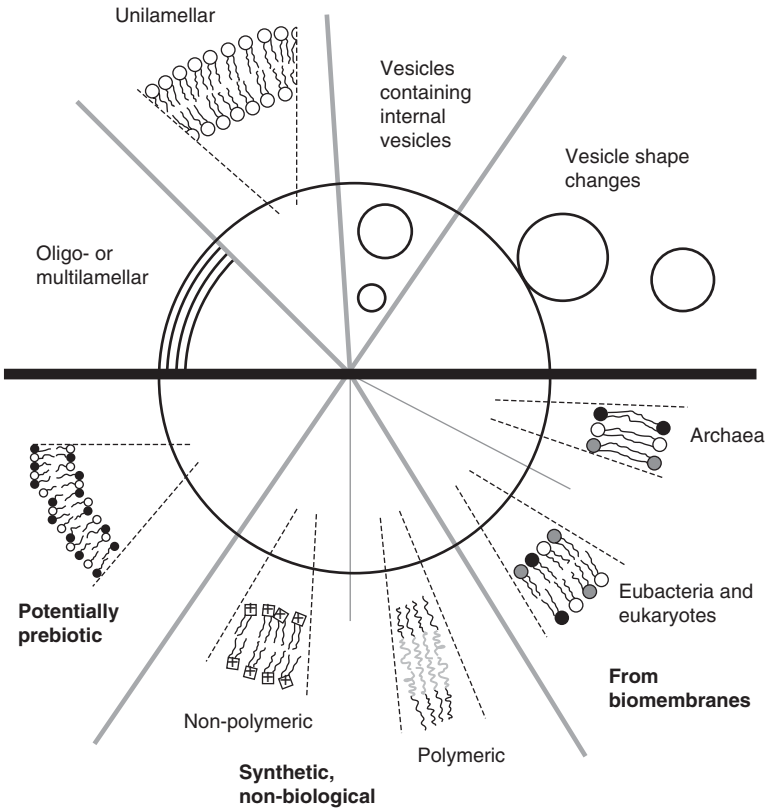

Figure 1. Diversity of morphology and composition of self-assembled vesicles. *Top*: Schematic representation of possible morphologies and shape changes. Vesicles may be multi-, oligo-, or unilamellar. They may also be multivesicular (containing smaller vesicles inside a large vesicle). Under certain conditions and for certain amphiphiles, vesicle shape changes can be induced (e.g., leading to vesicle budding and fission). Vesicles may also be nonspherical (e.g., tubular). The diameter of vesicles may vary between about 30 nm and more than 100 μm. *Bottom*: Vesicle formation occurs for a large number of chemically diverse amphiphiles, including those naturally occurring in biomembranes as well as completely synthetic amphiphiles. Of particular interest are single-chain amphiphiles (or mixtures of amphiphiles) that are potentially prebiotic.

use the plasma membrane to separate the cell's interior from the outside environment. The plasma membrane is composed of roughly equal parts protein and lipid amphiphiles, so one might assume that a protocell membrane was also composed of amphiphilic lipids and/or peptides. However, for simplicity, most experimental work thus far has focused on vesicles made of only one or two prebiotically plausible components (e.g., fatty acid). Vesicles can be made using many different types of amphiphiles, either naturally occurring or synthetic (Fig. 1, bottom). Because of the general robustness of the formation of vesicles, this process has been called an "archetype of self-assembly" (Antonietti and Foerster 2003).

In this article, we first review some fundamentals of self-assembly and focus on important features of vesicles made from single chain amphiphiles. For further discussion of vesicles as model prebiotic experimental systems, we refer the reader to reviews already in the literature (Mansy 2009; Monnard and Deamer 2003; Walde and Ichikawa 2001; Walde et al. 2006) and references contained within these reviews. We then turn to recent results and current challenges in research related to vesicles in the origins of life.

BACKGROUND

Thermodynamics of Self-Assembly of Amphiphiles

Self-assembled structures of amphiphiles are the result of a balance of attractive and repulsive forces. Amphiphiles tend to aggregate because of the hydrophobic effect, which stems primarily from the strong attraction of water for itself (Tanford 1973). Nonpolar solutes disrupt the isotropic hydrogen bonding of water, causing an entropic loss at the solute-water interface. Therefore nonpolar molecules tend to aggregate to minimize the interface (direct attractive interactions, such as van der Waals forces, play a relatively small role). In the absence of a repulsive force, the hydrophobic effect would lead to bulk-phase separation. However, repulsion among amphiphiles resulting from sterics (e.g., among hydrated head groups) or electrostatics favors the formation of structured assemblages.

A minimum number of molecules is required for effective reduction of the water–hydrophobic surface interface in an aggregate relative to free solution. This leads to a highly cooperative transition in which the concentration of aggregates increases dramatically near a critical aggregation concentration (cac) and for micelles, the critical micellization concentration (cmc). This process is often modeled as a phase transition; although such models are not strictly correct, they often capture the essential behavior of the system within experimental error. This approximation is quite good when aggregates are large (i.e., containing >50 molecules), as is the case for most micelles and vesicles.

Micelles are self-assembled structures with a liquidlike hydrocarbon interior and polar headgroups on the exterior (Fig. 2A). Several geometries are possible, including spherical, cylindrical, and ellipsoidal. The free energy of transferring an amphiphile from water into a micelle depends linearly on chain length, so the cmc depends exponentially on chain length (Tanford 1973). Vesicles are closed bilayer structures with an aqueous compartment (Fig. 2B). Whether micelles or vesicles are formed can be explained largely by packing considerations (Israelachvili et al. 1976; Israelachvili et al. 1977). The balance of attractive interactions and repulsive interactions gives an optimal interfacial area per molecule. Amphiphiles self-assemble into structures having area exposed to solvent (a) close to this optimum. Spherical micelles have the greatest interfacial area, in which $v/a = l/3$ (where v = volume and l = hydrophobic chain length), cylindrical micelles have $v/a = l/2$, and vesicles have $v/a = l$. To a first approximation, the presence of two chains on an amphiphile doubles v whereas the optimal a and l are constant, so a consequence of these considerations is that single-chain amphiphiles tend to form micelles whereas double-chain amphiphiles tend to form cylindrical micelles or vesicles. Vesicle formation is also favored by factors that reduce the optimal interfacial area. For example, fatty acids (prebiotically plausible

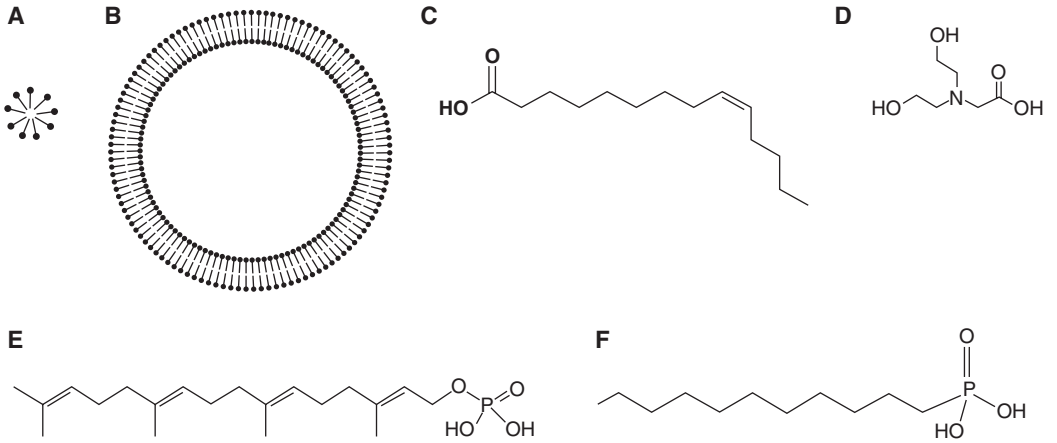

Figure 2. Structures of prebiotically plausible single chain amphiphiles and a commonly used buffer. (*A*) micelle; (*B*) vesicle; (*C*) myristoleic acid; (*D*) bicine; (*E*) geranylgeranyl phosphoric acid; (*F*) *n*-decylphosphonic acid.

single-chain amphiphiles) form micelles at high pH when their head groups are negatively charged, but they form vesicles at lower pH when their head groups interact more favorably (Gebicki and Hicks 1973).

Packing constraints also imply a minimum chain length required to form vesicles; for fatty acids (a single chain amphiphile), this length is around eight carbons (Monnard and Deamer 2003). Organic extracts of the Murchison meteorite (Lawless and Yuen 1979) show that abundances of fatty acids decreased as chain length increases, with a three-carbon increase corresponding to a ~10-fold drop in abundance. Roughly speaking, this drop in abundance is somewhat compensated by the decrease of cac as chain length increases (a three-carbon increase corresponds to a ~10-fold decrease in cac) (Chen et al. 2006). Although the details of these relationships depend on the synthetic pathway and buffer conditions, this general pattern suggests that, during prebiotic synthesis, each fatty acid would reach its cac at around the same time, leading to a relatively sharp transition to a vesicle world.

Kinetics of Self-Assembly of Amphiphiles

The kinetics of nucleation and growth of micelles are generally quite fast. Nucleation occurs on the order of microseconds to milliseconds, whereas exchange of monomers through the aqueous phase occurs on the order of nanoseconds to microseconds. These timescales depend on the association and dissociation rates of amphiphiles. As chain length increases, dissociation rates decrease (for alkyl sulphates, roughly 10-fold per three-carbon increase), whereas association rates decrease only slightly because of slower diffusion (within one order of magnitude from C6 to C14) (Aniansson et al. 1976; Hunter 2001).

Vesicle formation from a solution of micelles appears to have two relaxation times, a rapid mixing or aggregation of micelles, followed by slower growth and closure to form vesicles. For a mixture of anionic and zwitterionic surfactant micelles, the micelles mix within a few milliseconds and then coalesce into disclike micelles that grow and close into vesicles within a second (Weiss et al. 2005). For vesicles formed by increasing the pH of a solution of fatty-acid micelles, aggregation of micelles occurs very quickly (<12 ms) whereas relaxation into vesicles takes seconds to minutes (Bloechliger et al. 1998; Chen and Szostak 2004a). The kinetics of fatty-acid vesicle formation are particularly interesting with regard to the origins of life for two reasons: (1) the reaction can be autocatalytic, i.e., the presence of vesicles accelerates the formation of more vesicles (Walde et al. 1994b), and (2) vesicle

formation is catalyzed by many types of surfaces (Hanczyc et al. 2003; Hanczyc et al. 2007a), including montmorillonite, which also catalyzes RNA polymerization from activated monomers (Ferris and Ertem 1992). Once formed, closed vesicles can be fairly stable as supramolecular structures, surviving for several days or longer, even if their components exchange relatively rapidly.

Amphiphiles for Protocells

The primary components of modern cells are double-chain amphiphiles, particularly phospholipids, which consist of a headgroup and two acyl chains. Because of their geometry, double-chain amphiphiles form stable vesicles very readily. However, they pose several fundamental problems for a protocell, which result from slow dynamics. Phospholipids are relatively impermeable to charged compounds (e.g., K^+ permeability coefficient: 10^{-10} to 10^{-12} cm/s (Paula et al. 1996)), possibly because the low mobility of amphiphiles between the leaflets prevents facilitation of transport. Slow flip-flop would also limit vesicle growth by incorporation of new lipid (which requires redistribution of lipid from the outer to inner leaflet), inhibiting a key feature of a self-reproducing system. Modern cells treat their membranes as relatively static barriers, using flipases and permeases to mediate flip-flop and transport, respectively.

On the other hand, single-chain amphiphiles appear to be excellent candidates for prebiotic protocells for several reasons. They have relatively high permeability to ions and small molecules (e.g., K^+ permeability coefficient: 10^{-6} cm/s (Chen and Szostak 2004b)), presumably due at least in part to increased mobility of amphiphiles flipping between the leaflets, facilitating ion passage and circumventing the need for specific transporters. Fast flip-flop also permits vesicle growth through addition of an amphiphile feedstock to the vesicle exterior (Berclaz et al. 2001; Chen and Szostak 2004a). Single-chain amphiphiles also have high dissociation rates and relatively high water solubility, so the membrane components can be exchanged among vesicles rapidly, allowing redistribution to "growing" protocells (Chen et al. 2004). The cac is higher for single-chain lipids compared with double-chain lipids of the same chain length and similar head group structure, yielding a greater reservoir of monomers in solution. A limitation of single-chain amphiphiles is that they often form micelles, depending on the molecular geometries and buffer conditions, so the amphiphiles considered for prebiotic vesicles are constrained to have relatively small headgroups compared with double-chain amphiphiles.

Although we do not know which type of amphiphiles constituted the membranes of protocells, much attention has focused on fatty acids (Fig. 2c) and mixtures of fatty acids with their derivatives (e.g., fatty alcohols, amines, and monoacylglycerols). Fatty acids can be synthesized abiotically in several ways. For example, Miller-Urey-type electrical discharge reactions in a solution of ammonia under a nitrogen and methane atmosphere yield fatty acids with a chain length up to C12 (Allen and Ponnamperuma 1967; Yuen et al. 1981). Abiotic syntheses generally yield decreasing amounts of fatty acids of longer chain lengths. A synthesis simulating hydrothermal vents yielded fatty acids up to C33, from an aqueous Fischer-Tropsch-type synthesis using a heated solution of oxalic acid (which disproportionates into H_2, CO_2, and CO) (McCollom et al. 1999; Rushdi and Simoneit 2001).

Direct evidence for the abiotic presence of fatty acids comes from the detection of fatty acids in the interior of the Murray and Murchison carbonaceous chondrite meteorites from Australia (up to C8), as well as an Asuka carbonaceous chondrite meteorite (A-881458) from Antarctica (up to C12) (Lawless and Yuen 1979; Naraoka et al. 1999; Yuen et al. 1984; Yuen and Kvenvolden 1973). Fatty acids are relatively abundant in these meteorites, being 20 times more abundant than amino acids in the organic extract of A-881458. Indeed, organic extracts from the Murchison meteorite form boundary membranes when rehydrated (Deamer 1985; Deamer and Pashley 1989). The presence of fatty acids is particularly suggestive because

the chemical composition of these meteorites is believed to resemble that of the early solar system.

Depending on the solution pH, fatty acids self-assemble into different structures (Cistola et al. 1988; Small 1986). At low pH, the molecules are protonated and uncharged, resulting in an oil phase. At high pH, the molecules are deprotonated and negatively charged, resulting in the formation of micelles with a hydrophobic core and surface-exposed carboxylates that repel one another. Although the pK_a of a carboxylic acid is typically 4–5, the self-assembly of fatty acids leads to a cooperative effect that increases the pK_a. For example, oleic acid (C18:1) monomers have a pK_a of 4.5, but oleic acid assembled into a bilayer membrane in bicine buffer (Fig. 2D) has a pK_a of 8.5. Medium- and long-chain fatty acids incorporated into membranes have pK_as in the general range of 7–9 (Cistola et al. 1988). When the solution pH is near the pK_a, fatty acids assemble into bilayer membrane vesicles that are capable of entrapping solutes (Apel et al. 2001; Walde et al. 1994a). These vesicles have a net negative charge, with a formal surface charge density close to half of the molecular density of the membrane. As a result, cations are also associated with the surface, forming an electrical double layer (Grahame 1947; Hunter 2001). Several articles describe in detail the characteristics and limitations of fatty-acid systems (Deamer and Dworkin 2005; Mansy 2009; Monnard et al. 2002; Morigaki and Walde 2007; Walde et al. 2006).

Another category of single-chain amphiphiles that may be promising as a model for prebiotic vesicles uses a phosphate headgroup, such as the polyprenyl phosphates (e.g., geranylgeranyl phosphate; Fig. 2E). These amphiphiles can self-assemble into vesicles (Ourisson and Nakatani 1994; Pozzi et al. 1996). In an interesting parallel to fatty-acid membranes, which can be stabilized to pH changes by acyl alcohols (Monnard et al. 2002), polyprenyl phosphate membranes are stabilized by addition of polyprenyl alcohols (Streiff et al. 2007). Other phosphate-based systems under investigation include mono-*n*-alkyl phosphates and phosphonates (Walde et al. 1997); alkyl phosphonates in particular have been observed in organic extracts from the Murchison meteorite (e.g., decylphosphonate; Fig. 2F) (Cooper et al. 1992).

RECENT RESULTS

Membranes and Nucleic Acids

Vesicles could play a simple role as compartment boundaries for ribozymes (Chen et al. 2005), but they may contribute to an early cell in other ways as well. Because of the importance of the RNA world during origins of life, several groups are studying direct or indirect interactions between vesicle membranes and RNA. One mode of direct interaction between RNA and cationic vesicles is electrostatic attraction to form nucleic acid-lipid complexes (Thomas and Luisi 2005). Such complexes may also be formed with neutral or zwitterionic vesicles under the right buffer conditions (e.g., high divalent cation concentration). For anionic amphiphiles, the electrostatic repulsion between RNA (poly-U) and the vesicles can be overcome by covalently decorating the phopholipid headgroup with adenosine (Milani et al. 2007), analogous to the annealing of two complementary, negatively charged nucleic acid strands. Conversely, the nucleic acid can be conjugated to a hydrophobic moiety, such as cholesterol, which inserts into the membrane and brings the nucleic acid into the proximity of the vesicle (Banchelli et al. 2008). RNA aptamers for membranes have also been found by in vitro selection for RNAs that bound to phospholipid vesicles. These sequences appear to bind the membrane as aggregates (Janas and Yarus 2003). One such sequence was modified to include an RNA motif for tryptophan binding, and together these membrane-binding RNAs had a small but measurable effect on the rate of tryptophan permeation through the membrane (Janas et al. 2004).

Membrane vesicles can also have surprising consequences for the evolution of the RNA world. Membranes can accelerate the polymerization of RNA mononucleotides or amino acids during drying and wetting cycles (Deamer

et al. 2006; Rajamani et al. 2008; Zepik et al. 2007). This effect may be driven by an increase in local concentration of monomers, through trapping of monomers between lamellae during drying and mass action from dehydration. Although the mechanism is unknown, the liquid crystalline structure of the dried lamellae is believed to be important for this effect.

On the theoretical side, fresh efforts have been made in the analysis of polymerization dynamics relevant to nucleic acids ("prelife"). This mathematical analysis describes the distribution of sequences with and without replication and the occurrence of a phase transition among these regimes (Manapat et al. 2009; Nowak and Ohtsuki 2008). One interesting direction for this research would be consideration in the context of a protocell, to determine how small numbers and competition among protocells might affect the prelife dynamics.

Systems-Level Properties of Protocells

How did interactions arise between the membrane and its encapsulated contents? Two interactions have been described based solely on the physicochemical properties of the system. The growth of vesicles consists of the incorporation of fatty-acid molecules into the outer leaflet of the membrane, followed by the flip-flop of about half of these molecules into the inner leaflet. Fatty acid flip-flop into the inner leaflet will also transport protons (Hamilton 1998; Kamp and Hamilton 1992), so growth transduces the energy from the micelle-vesicle transition into a pH gradient across the membrane (Chen and Szostak 2004b). However, proton gradients across fatty-acid membranes only last as long as cations are not transported in the opposite direction, so a cell would need to use the energy stored in the proton gradient quickly before it decayed (Kamp and Hamilton 1992; Zeng et al. 1998). Regardless, the capture of energy is one way the growth of one component, the membrane, could confer an advantage to another component (encapsulated contents).

The emergence of the cell can also be approached from the perspective of the encapsulated genome. A transmembrane gradient of any impermeable solute (Δc) exerts osmotic pressure on a semipermeable membrane ($\Delta\Pi = n\Delta cRT$ for small solutes in dilute solution, where n = number of particles into which the solute dissociates). A charged solute generates more osmotic pressure, and the osmotic pressure of a charged polymer, such as RNA, will be due primarily to the counterions associated with it, in accordance with the Gibbs-Donnan equilibrium (Bartlett and Kromhout 1952; Tinoco et al. 2002). The replication of a genomic polymer would increase the osmotic pressure in a vesicle (assuming that the monomers equilibrate across the membrane). The resulting areal strain favors membrane growth, which relieves the strain by increasing the total area of the membrane. For amphiphiles with high rates of monomer exchange and flip-flop, like fatty acids, this leads to a competition among vesicles for amphiphiles, translating the fitness of the encapsulated replicase into the fitness of the whole cell (Chen et al. 2004). Darwinian competition between "cells" could have emerged simply on encapsulation of replicases or metabolic cycles inside vesicles; empty vesicles and vesicles encapsulating less efficient systems would shrink by losing membrane.

This competition has interesting implications for the natural selection of chemical traits in the genome and membrane. Because counterions cause most of the change in osmotic pressure after polymerization, protocells containing charged polymers as the genetic material, such as RNA or DNA, would outcompete those with neutral polymers (e.g., peptide nucleic acid). With respect to the membrane, the protocellular competition might favor the evolution of an enzyme that stabilized the membrane against loss of amphiphiles, such as by condensing two fatty-acid molecules together. In turn, the reduced permeability and growth dynamics of stabilized membranes might drive the coevolution of transporters and more enzymes. Thus the competition arising from the simple physical properties of an encapsulated replicase could result in a "snowball" effect on the evolution of cellular complexity. Such predictions may suggest further experimental exploration.

Computer Simulation of Protocell-Like Systems

In an era of increasingly powerful and accessible computation, molecular dynamics (MD) simulation is a promising technique for exploring our understanding of the behavior of relatively large chemical systems like vesicles. In general these simulations use a combination of empirical and *ab initio* information to describe energy potentials for molecular conformations and intermolecular forces (dispersion and electrostatic forces) and solve the Newtonian equations of motion numerically. The size of a vesicle system is roughly hundreds of thousands to a few millions of atoms (e.g., a single fatty acid is roughly 50 atoms, and thousands of fatty-acid molecules would compose a small vesicle, and water would also compose a substantial mole fraction of the system). Currently an MD simulation of this size could simulate about 100 nanoseconds, which would be enough to study the fast kinetics of self-assembly. An atomistic simulation of dipalmitoyl phosphatidylcholine (DPPC) in water recapitulated vesicle self-assembly (de Vries et al. 2004). In addition to testing our understanding of the system, ideally these simulations could be useful to experimentalists for building biophysical intuition and emphasizing features to be further explored. For example, the simulation of a DPPC vesicle indicated a large asymmetry in mass and ordering between the inner and outer bilayers, suggesting a potential mechanism for asymmetric insertion of molecules into the membrane.

Slower dynamics or larger systems could be modeled using coarse-grained rather than atomistic simulations (Klein and Shinoda 2008). One interesting but poorly understood phenomenon that might benefit from MD simulation is the recent observation that dried lipid membranes accelerate the polymerization of amino acids and nucleotide monophosphates, perhaps by preorganizing the reactants into the reactive geometry (Rajamani et al. 2008).

Another style of simulation of protocells focuses on the reaction (or reaction-diffusion) kinetics of the system without modeling the molecular details. Although much work, such as Manfred Eigen's classic model of hypercycles (Eigen 1971), dealt with metabolic cycles without explicit reference to a membrane, recently the potential for interactions between the membrane and encapsulated metabolic reactions has come under closer scrutiny (Mavelli and Ruiz-Mirazo 2007). Stochastic simulations are of particular interest because the volume of a small vesicle could be less than one attoliter, such that micromolar concentrations would give only a handful of molecules per vesicle. Although care is always required when dealing with assumptions in a model, simulations can provide a testing ground for our understanding, and may become a useful tool for understanding experimental results and generating interesting, experimentally testable predictions in protocell-sized systems.

CHALLENGES AND FUTURE DIRECTIONS

Physical Forces and Protocells

How would protocells interact with their environment? Although some interest has focused on interactions at a molecular level, such as the permeability and thermostability of vesicles (Mansy et al. 2008; Mansy and Szostak 2008), less work has been performed to relate colloidal processes to protocells. For example, transport phenomena might give us clues about protocell movement through a heterogeneous environment or even initially homogeneous environment. Hanczyc et al. showed how oleic anhydride oil droplets added to an alkaline solution spontaneously move, as exothermic hydrolysis of the anhydride sets up a convective current within the droplet; the direction of motion appeared to be the outcome of symmetry-breaking fluctuation (Hanczyc et al. 2007b). The theory of osmophoresis suggests that vesicles in a solute gradient will move in the direction of lower solute concentration (Anderson 1983; Nardi et al. 1999). Such effects, if experimentally relevant, could inform our understanding of how a protocell would move within its environment as a consequence of colloidal forces, without motor machinery.

Entropy might play a very interesting role in protocells. For example, theory predicts that two chromosomes in a cell tend to segregate from each other to maximize conformational entropy (Jun and Mulder 2006). This finding suggests that protocell genomes might not require extra machinery to segregate its genetic material. Along similar lines, cell division machinery (in particular, a contractile ring) was recently discovered to be unnecessary for reproduction and division of *Bacillus subtilis* (Leaver et al. 2009). The observed mode of division is reminiscent of a physical phenomenon, the Rayleigh instability (also known as "pearling" in tubular liposomes), lending credence to the idea that protocells would not require division machinery (Chen 2009). One might also expect protocells to interact with one another through entropic effects, such as the depletion effect, in which the entropy of the aqueous solution is maximized when large particles are close together, resulting in an effective attraction between the particles. Such effects could be interesting as leading to assembly of protocells into larger structures. Investigation of the role of physical forces in protocells may present many opportunities for collaboration between traditional soft condensed matter physicists and origins of life researchers.

Vesicles from Mixtures

Studies on vesicles formed from potentially prebiotic amphiphiles have so far been limited to systems containing one or two types of amphiphiles. This is in contrast to the output of simulated prebiotic chemical reactions, which typically produce very heterogeneous mixtures of compounds. In addition, little experimental work has been performed to integrate peptides and lipids. (A notable exception to this bias toward working with one or two component systems was a study using the heterogeneous organic extract from a carbonaceous chondrite meteorite to form vesicles [Deamer 1985].) The reason for this bias is mainly the avoidance of analytical difficulties, but clearly it is not very realistic.

What properties might vary with membrane composition? Several features characterize a particular vesicle system and differentiate it from others, including (1) lamellarity, (2) average membrane thickness and its fluctuations, (3) amphiphile dynamics in the membrane, (4) membrane permeability, i.e., the ability to keep solutes captured inside the vesicles and to stabilize concentration gradients across the membrane, (5) chemical stability of the amphiphiles, (6) membrane surface charge, (7) membrane fluidity, (8) possibility of membrane budding and fission (leading to vesicle divisions), (9) vesicle aggregation or vesicle adsorption to surfaces, (10) vesicle fusion, (11) colloidal stability at different temperatures and bulk solution compositions. All these properties ultimately depend on the chemical structure of the amphiphiles, the environmental conditions, and the process of vesicle formation. The process of vesicle formation is relevant because most vesicles are kinetically trapped systems (i.e., not at true thermodynamic equilibrium). Lamellarity and size depend strongly on how the vesicles are prepared, but the other features depend primarily on the vesicle composition and buffer conditions.

There are challenges to using vesicles based on fatty acids as protocell models (Monnard and Deamer 2002). One limitation for practical studies is the failure in preparing vesicles from long-chain fatty acids at room temperature, for example from hexadecanoic acid, because of the high melting temperature of saturated linear hydrocarbon chains. According to the phase diagram, a lamellar phase exists at 70°C (Skurtveit et al. 1989). However, recently it was shown that vesicles form from long-chain fatty acids at room temperature if guanidine hydrochloride is added (Douliez et al. submitted for publication). This illustrates how vesicles of mixed composition can actually have unanticipated properties beneficial to a protocell, such as robustness to ionic strength, lowered cac, or even propagation of information (as discussed later) (Monnard and Deamer 2003). One of the challenges for the future will be to further study these mixtures to understand the chemical basis for their effects on vesicle characteristics. Other mixtures to explore include mixtures of fatty acids with small molecule components, mixtures of different types

of fatty acids, and mixtures of fatty acids with peptides.

Self-Assembled Structures and Information

The storage and propagation of information is a key feature of biological systems. Nucleic acid sequences fulfill this requirement in a straightforward fashion, particularly in the case of ribozymes, where the informational material itself has chemical activity. However, in a very broad sense, molecules other than nucleic acids carry information about their reactivity and physicochemical properties (Graham et al. 2004). Self-assembling molecules contain the information for building supramolecular structures, such as vesicles and micelles. For example, potassium decanoate, $CH_3(CH_2)_6COO^-K^+$, carries information on the thermodynamics of its self-assembly behavior. When dissolved in water at a concentration of 29.1 g/L (150 mM), micellar aggregates form (Namani and Walde 2005). Each micelle contains a hydrophobic core that allows some solubilization of molecules that are not soluble in water, so the self-assembly of the amphiphilic decanoate molecules confers new physicochemical properties to the aqueous solution (Luisi 2002). Could self-assembled, noncovalent systems also propagate their "information"?

An interesting model addressing this question was proposed recently (Segre et al. 2000). In a solution containing a mixture of amphiphiles, if amphiphiles of a certain type favored the incorporation of other amphiphiles in a selective fashion, an assemblage would reach a particular steady state whose composition contains some information. In simulations they observed that abrupt transitions could occur among different steady states, suggesting that different compositions could coexist and thereby provide variation on which natural selection might act. An experimental system corresponding to this model would require determining the on- and off- rates of different amphiphiles in vesicles spanning a range of compositions. A particularly interesting technical challenge associated with this theoretical problem is the identification of the composition of an individual vesicle.

Self-assembly as a Quantitative Trait

Although one working definition of life (a self-sustaining chemical reaction capable of Darwinian evolution) was given in the introduction to this article, alternative definitions also exist, often in the form of several criteria. The difficulties encountered in defining life suggest that life is a quantitative, not qualitative, trait, which could be measured by an appropriate metric. Chirikjian and coworkers developed metrics for the degree of self-replication and task complexity in a framework of self-replicating robots (Lee et al. 2008). For example, some robots copy themselves by joining two already complex building blocks (low self-replication) in a highly structured environment with many landmarks (low task complexity), whereas other robots copy themselves by joining several simple building blocks (high self-replication) in an unstructured environment (high task complexity). These calculations require knowledge of the components, their interconnections, and the environment. Application of this quantitative approach to chemical systems and to other properties (i.e., evolvability and life) poses an interesting intellectual challenge that might also help integrate our understanding of different model systems of protocells.

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