Liposome-Mediated Biomolecular Computation *1

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Abstract

Biological vesicles are composed of a phospholipid membrane bilayer, which has folded on itself to form a spherical structure with an aqueous cavity. We propose that synthetic vesicles, termed liposomes, composed of specific species of phospholipids might provide a useful architecture for the execution of some existing algorithms for DNA based computation. In this model, the liposomes would serve simultaneously as water-filled chambers containing specific DNA reactants; and as valves to permit, via controlled fusion, interaction of only the DNA molecules and enzymes appropriate for a particular stage of computation.

1. Introduction

One of the greatest potentials of DNA based computation is to carry out massively parallel operations. In order to exploit fully this potential, these parallel operations should be performed on different subsets of molecules, with the resultant strings all eventually combined into one solution tube. To address the technological problem of differentially affecting desired vs. undesired interactions of DNA reactants, Reif et al have recently proposed a Micro Flow Bio-Molecular Computation (MF-BMC) architecture. In the MF-BMC model, a decrease in human intervention is brought about through automation of miniature chambers, pumps, valves and sensors [6]. Kurtz et al and Hagiya have previously proposed that biological membranes might prove useful in forming compartments for organizing DNA based computation [8], [9]. We present a model based on this idea, termed Liposome-Mediated Biomolecular Computation (LMBMC). The fundamental concept underlying LMBMC is that liposomes could serve simultaneously as chambers and valves for directing DNA based computation. Our preliminary analysis of this model suggests that, for certain types of computation, this approach might provide a useful alternative to MF-BMC.

2. Biochemistry of Biological Membranes

Cells employ biological membranes to separate their internal contents from the surrounding environments [10], [20]. Intracellular organelles use membranes to further compartmentalize specific molecules and processes. Lipids, mainly phospholipids and sphingolipids, are the primary contributors to biological membrane structure. Membrane lipids have a polar or ionic (water-loving) head group and two or more hydrophobic (water-hating) acyl chains. As Figure 1 illustrates, when the cross sectional area of the head group (dark circles) is equal to the area of the tails (light chains), each lipid will adopt a cylindrical shape. In aqueous solutions, the tails will avoid high-energy interactions with water by aggregating together forming a two-dimensional bilayer. If the bilayer is large enough, it will fold on itself and form a three-dimensional, spherical vesicle enclosing an aqueous cavity.

In addition to lipids, biological membranes are composed of peripheral and integral proteins (often attached to lipids or sugar moieties) and cholesterol. The presence and amount of these constituents determine the

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function or behavior of the liposome. Since the membrane is held together by weak, as opposed to covalent interactions, all components are free to diffuse laterally in the two-dimensional plane. The stable, flexible nature of biological membranes allows one liposome to undergo either fission, splitting into two compartments, or fusion, which combines two liposomal cavities into one large compartment. Finally, the hydrophobic region within the bilayer restricts the passage of large polar solutes. Thus, membranes are semi-impermeable.

Figure 1. Lipids associate to form a bilayer (left). The bilayer can fold on itself to form a liposome with an aqueous cavity (right). From Lehninger et al, 1993, [10].

3. Advantages of LMBMC

Computation using this type of experimental system, would have the following advantages:

3.1 Cost. The components of artificial membranes (both lipids and proteins) are inexpensive and available in large, pure quantities. Furthermore, manipulation of membranes does not require equipment other than that found in a typical molecular biology laboratory.

3.2 Synthesis. The formation of artificial liposomes has been researched extensively. For example, well-characterized methods exist which produce one large unilamellar vesicle, which is then divided into hundreds of identical small unilamellar vesicles or liposomes. These liposomes can then be separated by gel filtration, without disrupting membrane integrity, to provide a homogenous preparation. Synthesis can be performed in one reaction solution in a matter of hours [17].

3.3 Diversity. Liposomes can be synthesized with various membrane phospholipids, integrated proteins and entrapped solutes. In addition, like all organic molecules, membrane phospholipids can be covalently attached to antibodies, magnetic components or fluorophores. By combining all of these factors, one can generate a finite set of liposomes, each with its own topologies, behaviors and characteristics [18].

3.4 Shelf Life. When a solution of liposomes is produced, it can be stored for weeks, with little spontaneous fusion or rupture.

3.5 Achievement of High Initial Concentration. When preparing liposomes, solutes up to $1 \times 10^6$ Da (DNA strands up to 3000-4000 bases) can be enclosed at the same concentration that they appear in the starting solution [18]. Therefore, one can produce a high initial concentration of internal solutes. This is important, because after many traditional DNA computation operations, the effective concentration of reactants can become homeopathically low [9]. Conversely, when two liposomes of equal size fuse, the concentration of entrapped solutes increases. Following fusion, the surface area increases by a factor of 2,
while the volume increases by a factor of $2\sqrt{2}$. As a consequence, the new surface area cannot hold the initial contents and some water is excluded, increasing the internal concentration [18].

3.6 Specificity. Specificity is the key to any biological or DNA computing operation. By varying the membrane lipid and protein content of liposomes, one can direct the behavior (fusion or other manipulations) of a distinct subset of the liposome population.

3.7 Parallelism. There are a number of physical limitations to the amount of connectivity between Reif’s MF-BMC chambers and channels [6], because of their solid architecture. By contrast, if liposomes are synthesized correctly, a very large number of parallel fusion “connections” (merging of liposome contents) can form in solution. This is because the liposomes are free in solution, so that every liposome can potentially interact with all others. On the other hand, there are also limitations on the rates of parallel processes with liposomes, since the rate of fusion in diffusion-limited.

3.8 Thermostability. Many unicellular organisms have evolved to withstand a wide variety of harsh environments, such as extreme temperatures. Thermophiles can employ enzymes with high temperature optimal activities, long-chain fatty acids and special proteins in their membranes to thrive in 85 to 95°C environments [11]. It is thus possible to synthesize liposomes with thermostable membranes, permitting high temperature DNA computation operations such as melting and PCR, to occur without destroying the liposomes.

3.9 Automation. As described in the following section, most of the proposed LMBMC operations can be readily automated.

4. The Operations

A set of liposomes is analogous to a set of microscopic test tubes. Thus, most novel operations will involve the micromanipulation of T, as opposed to specific sequences, c. (As defined in [2])

4.1 Modified or Novel Operations for LMBMC

4.1.1 Initialiation. This LMBMC operation involves the synthesis of liposomes containing appropriate integrated proteins and entrapped solutes, including both DNA strands and enzymes. The initialized vessel would contain all of the different synthesized liposomes.

4.1.2 Liposome Merge/Union. This is the most important operation in LMBMC. It is traditionally defined as the combination of two or more sets of strings. This operation is very common in solution-based DNA computation, and is also error prone, since DNA strands can be lost in the process. Merging of strands in LMBMC is accomplished by one of three different mechanisms; lipid controlled fusion, protein mediated fusion or viral insertion, as follows:

4.1.2.1 Lipid-Controlled Fusion. The most attractive mechanism for the Merge/Union operation is lipid-controlled fusion.

As depicted in Figure 2, a fusion event involves the aggregation of liposomes (A), interbilayer contact or lipid mixing (B and C) and fusion of aqueous contents (D). It should be noted that the specificity and rate-limiting step of fusion is dependent on the rate of liposomal aggregation. For example, liposomes composed of phosphatidylserine (PS) fuse with a $t_{1/2}$ (half-life) of days, while phosphatidylcholine (PC) liposomes are never allowed to aggregate or fuse, regardless of temperature or ionic conditions [5].

In lipid controlled fusion, simple physical/chemical changes in, for example, $[\text{Ca}^{2+}]$, $[\text{Mg}^{2+}]$, pH or temperature, can selectively induce fusion of liposomes that contain specific phospholipid components [4]. For example, if an algorithm required the fusion of liposomes A and B (in one step) and subsequently C, one would initialize A and B with pure phosphatidic acid (PA), and D with a 1:1 ratio
of PA:PC. It is known that PA liposomes selectively fuse in $[\text{Ca}^{2+}] = 0.2\text{mM}$, while PA:PC liposomes can fuse with pure PA liposomes at higher $[\text{Ca}^{2+}]$ [5]. The addition of $\text{Ca}^{2+}$ is a reversible manipulation (since it can be selectively precipitated), as are pH and temperature changes.

As Figure 3 illustrates, when a tree-like algorithm is required, phospholipid fusion rules, can be employed to devise a scheme for selective fusion at each level of the tree. Lipid-controlled fusion involves easy, hands-off manipulations amenable to automation. On the other hand, it appears the number of levels (depth) in a fusion-tree algorithm is limited, because after many levels, the phospholipid content of the growing liposome would become too complex, rendering it susceptible to side reactions and undesired fusion events.
4.1.2.2 Protein-Assisted Fusion. Biological cellular vesicles employ integrated proteins to assist in selective fusion. For example, studies performed both in vitro and in vivo have shown that membrane-integrated SNARE proteins are capable of docking or aggregating two specific liposomes together in preparation for fusion [13], [15]. Furthermore, aqueous external factors necessary for the fusion event (NSF and SNAPs) could be applied at the specific level where fusion is desired. There are numerous species-specific pairs of SNARES (types v and t SNARES), which can increase the combination of distinct fusion events. Thus, protein-assisted fusion may be combined with lipid controlled fusion to increase the possible complexity of a fusion-tree algorithm.

4.1.2.3 Viral Insertion. In the third LMBMC Merge/Union operation, viral insertion can be used to add a single string to a set of liposomal strings. Certain viruses are capable of encapsulating double stranded or single stranded DNA. In solution, the virus interacts with its receptor, inserting a single copy of its DNA into an accepting liposome [10]. This approach would only be useful if one copy of a strand is sufficient for a computation operation. Viral insertion and protein-assisted fusion are illustrated in Figure 4.

![Figure 4](image.png)

Figure 4. DNA contained in liposomes A and B can fuse via protein-assisted fusion, while one DNA molecule from virus C can merge with liposome B contents via viral insertion.

4.1.3 Filter. This is another novel LMBMC operation. By adding certain factors to solution, one can selectively (depending on phospholipid components) filter small solutes out of the liposomes, while retaining large ones. For example, Sphingomyelinase induces leakage of solutes in sphingomyelin-containing liposomes [1]. The peptide sticholysin II creates a pore of approximately 1 nm diameter, which allows solutes up to 1 kDa to exit sphingomyelin and PC-containing liposomes [3]. Finally, antibacterial cecropin-A-melittin hybrid peptides can slowly induce leakage of 20 kDa solutes in PS or PC liposomes [12]. The Filter operation could possibly be used to sieve small strands from the liposomes, that have not been integrated into longer solution strands (for example, in a constructive primer extension NP-Complete algorithm). As more selective and specific leakage-inducing agents are discovered, they would be integrated into LMBMC algorithms.

4.1.4 Liposome Separate. If one expects the solution molecule of a computation to reside in the largest liposome (following many fusion events), the Liposome Separate operation, achieved via gel filtration, could be used to separate and isolate liposomes according to size.

4.1.5 Fusion Detect. Since there are many assays available to confirm the fusion of two liposomes, a Fusion Detect operation can be defined. This would be employed to signal the completion of an algorithm (Figure 5), or the incorrect fusion of two liposomes. For example, if species A and B interact
to produce a visual or fluorescent signal, the final fusion event of a *fusion-tree algorithm* can be readily detected.

4.1.6 Lyse. Detergents can be added to solubilize all membrane lipids and proteins, releasing the DNA strands for purification or detection. This is defined as the *Lyse* operation.

4.2 Potential Application of Traditional Sequence-Specific DNA Computation Operations to LMBMC

Most operations described in the DNA based computation literature involve the identification or manipulation of a specific DNA sequence. These operations, *Append, Amplify, Ligate, Digest* and *Cut*, are all catalyzed by enzymes. In solution based DNA computation, enzymes (such as ligase, polymerase or exo/endonuclease) are simply added to test tubes. However, in LMBMC, sequence-specific manipulation would require fusion events that combine DNA/enzyme with new DNA. Alternatively, specialized *catalytic liposomes* could be fused with DNA-containing liposomes for specific operations.

4.3 Operations That Are Difficult To Achieve In LMBMC

The most commonly used operation that would be difficult with LMBMC is *Split*, since artificial liposome fusion is an irreversible event. Cellular processes do exist that induce budding or splitting of membrane contents into two compartments, but the factors involved, such as receptors, protein coats and signals, are probably too complex for use with LMBMC.

Unlike solution or surface-based computation, termination of the enzymatic operations described above cannot be achieved by physical means, such as washing or gel separation. Furthermore, traditional operations such as *Length, Detect and Test*, which operate exclusively on DNA strands, can only be executed after the *Lyse* operation. Finally, the *Amplify* (PCR) operation can be conducted only with thermostable liposomes. If these liposomes are used, it should be noted that information regarding selective fusion is not available. Therefore, amplification should also be performed after the final *Lyse* operation. Successive *Melt* and *Append* operations are acceptable, as the temperatures required should not disrupt liposome integrity.

5. Potential Applications Of LMBMC To Existing Algorithms

It seems likely that the LMBMC architecture described above will prove useful in a number of existing algorithms for DNA-based computation. Three such examples are described below.

5.1 State Transition Model (Hagiya et al). This seems the most attractive algorithm for application of LMBMC [16]. This group has experimentally demonstrated that Boolean expressions and NP-Complete problems can be successfully solved with their unique *successive localized polymerization* operation. Although this operation involves successive primer extension reactions, Hagiya et al have developed a new constant temperature protocol, which would allow the application of LMBMC, without extreme thermophile membrane components [7]. Solutions could be generated *intramolecularly*, in a massively parallel fashion, as the program, transitions and current state can reside on a single molecule. One limitation of the state transition model is efficient dataflow. Hagiya et al note that the size and complexity of their approach would be increased dramatically if the program and state resided on one molecule, while the inputs are encoded on others [8]. If the program were accomplished it could produce an output state, which would be passed on to other programs as input. It seems likely that, with LMBMC, simple *fusion-trees* would readily increase the dataflow and complexity of the state-transition algorithms developed by this group.

5.2 Self-Assembly DNA Computation Model (Erik Winfree). LMBMC appears to provide the ideal architecture to mediate large-scale application of this computation approach. It has been shown that NP-Complete solutions can be synthesized by the self-assembly of DNA tiles [19]. When N is large, the complexity of the solution increases to the point where the time required for solution generation is immense. However, if partial solutions were generated in enclosed liposomes, the entire population could be induced to slowly fuse into one large unilamellar vesicle, where all partial solutions could interact like completed subsections of a jigsaw puzzle. This would not be possible with a traditional solution-based Merge operation, because large, non-ligated solution arrays would be disturbed. Furthermore, as the liposomes fuse, the
reaction volume would increase, allowing room for larger structures to form, while the concentration of DNA would also increase, compensating for the increased complexity.

5.3 Boolean Circuit Algorithm (Ohigara and Ray). This approach [14] already employs a tree-like strategy. Unfortunately, each level requires a Length operation, which cannot be performed in LMBMC, until the completion of all liposomal fusion events. If this step were replaced with another operation (such as endonuclease Digest or exonuclease Destroy of wrong solutions), LMBMC might supply the ideal medium for their computation.

5 Conclusion

We have described here an LMBMC model for DNA based computation, based upon manipulations of DNA reactants enclosed in liposomes possessing specific chemical properties. We have considered briefly the possible application of this model to several known DNA based algorithms. However, further analysis, both theoretical and experimental, will be required to ascertain the specific liposome structures and manipulations required for the execution of each algorithm. It also seems likely that the model presented here can be extended to the development and application of new algorithms for DNA based computation, that are based specifically upon the particular strengths of LMBMC. It is also notable that conditions for specific phospholipid fusion (e.g., precise Ca$^{2+}$ concentrations required for fusion of specific lipids) have not previously been examined in detail, probably because there was no reason to do so. It thus seems possible that further experimental studies will reveal more specific information about the precise lipid combinations and environmental conditions that will lead to controlled fusion of liposomes. It may even prove possible to synthesize phospholipid head groups not found in nature, that would permit specific "fusion rules" not provided by natural phospholipids. We note finally that the experimental execution of a simple example of LMBMC would provide a strong "proof-of-principle" for this model.

References


