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Controlled Fusion of Synthetic Lipid Membrane Vesicles

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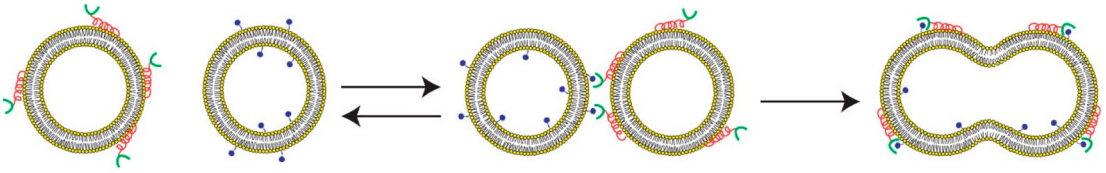

Lipid membrane fusion is a fundamental noncovalent transformation as well as a central process in biology. The complex and highly controlled biological machinery of fusion has been the subject of intense investigation. In contrast, fewer synthetic approaches that demonstrate selective membrane fusion have been developed. Artificial recapitulation of membrane fusion is an informative pursuit in that fundamental biophysical concepts of biomembrane merger may be generally tested in a controlled reductionist system. A key concept that has emerged from extensive studies on lipid biophysics and biological membrane fusion is that selective membrane fusion derives from the coupling of surface recognition with local membrane disruption, or strain. These observations from native systems have guided the development of de novo-designed biomimetic membrane fusion systems that have unequivocally established the generality of these concepts in noncovalent chemistry.

In this Account, we discuss the function and limitations of the artificial membrane fusion systems that have been constructed to date and the insights gained from their study by our group and others. Overall, the synthetic systems are highly reductionist and chemically selective, though there remain aspects of membrane fusion that are not sufficiently understood to permit designed function. In particular, membrane fusion with efficient retention of vesicular contents within the membrane-bound compartments remains a challenge. We discuss examples in which lipid mixing and some degree of veside-contents mixing is achieved, but the determinants of aqueous-compartment mixing remain unclear and therefore are difficult to generally implement. The ability to fully design membrane fusogenic function requires a deeper understanding of the biophysical underpinnings of membrane fusion, which has not yet been achieved. Thus, it is critical that biological and synthetic studies continue to further elucidate this biologically important process. Examination of lipid membrane fusion from a synthetic perspective can also reveal the governing noncovalent principles that drive chemically determined release and controlled mixing within nanometer-scale compartments. These are processes that figure prominently in numerous biotechnological and chemical applications. A rough guide to the construction of a functional membrane fusion system may already be assembled from the existing studies: surface-directed membrane apposition may generally be elaborated into selective fusion by coupling to a membrane-disruptive element, as observed over a range of systems that include small-molecule, DNA, or peptide fusogens.

Membrane disruption may take different forms, and we briefly describe our investigation of the sequence determinants of fusion and lysis in membrane-active viral fusion peptide variants. These findings set the stage for further investigation of the critical elements that enable efficient, fully functional fusion of both membrane and aqueous compartments and the application of these principles to unite synthetic and biological membranes in a directed fashion. Controlled fusion of artificial and living membranes remains a chemical challenge that is biomimetic of native chemical transport and has a direct impact on drug delivery approaches.

Introduction

Many designed membrane-active agents utilize biomimetic self-assembly of components within the lipid bilayer to open a pore, leading to depletion of transmembrane chemical gradients.[1] A complementary membrane transport reaction that is less investigated is selective membrane fusion. Often, the binding of pore-forming membrane-active agents to lipid membranes can trigger nonspecific

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vesicle aggregation and fusion when used at higher concentrations than typical for channel formation. It follows that it may be possible to create synthetic membrane-bound systems to mediate the selective merger of two membranes by combining directed surface recognition with a membrane-activating component. These studies provide a fundamental understanding of the chemistry of lipid assemblies and serve to support and develop new hypotheses regarding native membrane fusion systems. Furthermore, control of small vesicle fusion has potential biotechnological applications in delivery and nanocompartmentalized chemistry. We review herein the recent development of synthetic selective membrane fusion systems by our group and others.

Lessons from Native Membrane Fusion Systems

The synthetic fusion systems take inspiration from the wealth of studies on native fusion machinery. Both endogenous and viral membrane fusion machineries have been the subject of extensive study over the past few decades and have been found to exhibit remarkable broad-stroke similarities.[2−4] The native fusogenic drivers of viral host cell entry and endogenous neurotransmitter release are helical bundle proteins, suggesting the possibility of a "uniting mechanism".[2] The helical bundle fusion proteins of influenza and HIV undergo pH- or receptor-triggered rearrangements that expose a membrane-disruptive fusion peptide domain.[4,5] An endogenous cognate is found at the synaptic vesicle−plasma membrane interface, wherein vesicle docking and fusion is triggered in large part by the formation of in trans "SNARE" complexes, which are heterotetrameric, membrane-anchored coiled coils.[3] The components of the heterotetramer are split between the target and vesicular membranes, and their union results in the formation of a tetrameric helical bundle protein anchored in both the vesicular and target membranes. In conjunction with a host of regulatory and assisting proteins,[3,6] formation of the SNARE complex devices rapid membrane fusion. Unlike influenza and HIV, which use specific receptor-binding events to guide the apposition of the viral and host membanes, SNARE complex formation results in both selective apposition and membrane activation.[7,8] Though there are many molecular components of synaptic vesicle fusion, the membrane-anchoring peptides of the SNARE proteins have been found to cause indiscriminate membrane fusion on their own.[9] Overall, the general theme of coupled surface recognition and membrane activation resonates in native fusion systems.

Reductionist Synthetic Models of Membrane Fusion

Synthetic models of membrane fusion are highly simplified relative to native systems. While this detracts from their relevance to biological processes, more defined biophysical questions may be posed using artificial systems that are not possible in heterogeneous native systems. Thus, while the details of native fusion depend strongly on biological context, synthetic membrane systems reveal *possible* biophysical chemistry outcomes. One concept that may be probed using artificial membrane fusion systems is whether any combination of vesicle docking and activation leads to selective lipid bilayer fusion. Rothman and co-workers first demonstrated selective fusion of lipid vesicles using minimal SNAREpins embedded in synthetic large unilamellar vesicles (LUVs).[10] The components of the heterotetrameric SNARE helical bundle were divided and membrane-anchored into the synthetic vesicles. Combining the two vesicle populations resulted in lipid mixing, with one round of fusion completed in 20 min. Though this rate is considerably slower than fusion at a biological synapse, selective membrane fusion was clearly demonstrated. The minimal SNAREpin system contained the components of recognition and activation[11] and conformed with the lessons gleaned from studies on biological fusion. Inspired by the success of Rothman's system and other SNARE-derived synthetic fusion systems,[3] we considered other reductionist possibilities that did not utilize helical bundle recognition and activation to demonstrate the generality of this approach to selective membrane chemistry.

Vancomycin-Targeted Synthetic Vesicle Fusion

Small-molecule-guided membrane fusion was particularly appealing as a means to explore scope, as it has no native cognate. To this end, we considered known strategies for small-molecule recognition as well as membrane activation. One of the most well-studied small-molecule recognition events in water is the binding of vancomycin glycopeptide with D-Ala−D-Ala dipeptide.[12] Vancomycin, an antibiotic of last resort, is known to bind to the D-Ala−D-Ala dipeptide sequence and block its displacement in the transpeptidase-catalyzed cross-linking of the bacterial peptidoglycan cell wall. Well-precedented vancomycin modification strategies[12] facilitated the use of this antibiotic and its binding partner as fusogenic components (Figure 1). We chose magainin II, a positively charged 23-residue antimicrobial peptide, as the membrane-activating component of our system.[13,14] Magainin binds preferentially and disruptively to negatively charged membranes,[15] leading to membrane-lytic

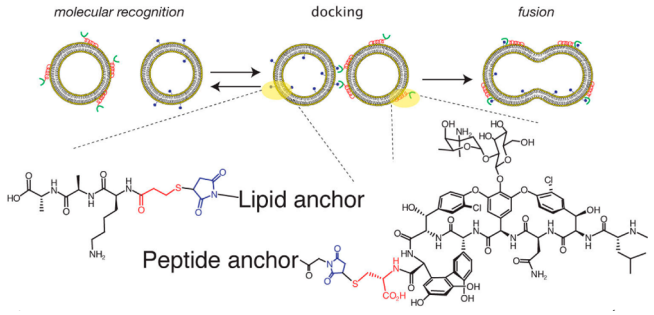

FIGURE 1. Illustration of selective vesicle fusion driven by recognition of the magainin–vancomycin (red coils) conjugate by lipid-attached D-Ala–D-Ala dipeptide (blue dots).

activity at moderate micromolar concentrations. Standard solid-phase peptide synthesis methods allowed convenient access to magainin N-terminated with an electrophilic maleimide for subsequent solution-phase coupling with vancomycin thiol derivatives. Sulfhydryl installation on vancomycin was accomplished either by selective reductive alkylation of the N-terminal secondary amine with 1,4-dithiane-2,5-diol or by C-terminal amidation with cysteine.[14] A lysine–D-Ala–D-Ala phospholipid binding partner (Kaa-POPE) for the magainin–vancomycin (MV) conjugate was synthesized in a similar way using tetraethylene glycol to link the peptide and lipid.

Negatively charged LUVs bearing MV conjugate were reacted with zwitterionic LUVs containing 1–2% Kaa-POPE, resulting in the docking of charged and neutral vesicle populations via vancomycin–Kaa binding. Docking was apparent by increased dynamic light scattering, which stabilized at a 50% increase. Immediate and full membrane fusion, as judged by FRET-based lipid mixing assays,[16] was observed upon mixing of the two LUV populations (Figure 2). Importantly, both the recognition and activation components were required for fusion, and the reaction could be blocked by competitive inhibition with free vancomycin. The fusion rate increased with the ratio of unlabeled to labeled vesicles as well as with the concentrations of lipid and peptide fusogens.[14] One round of fusion was complete in 20 min, similar to the synthetic SNAREpin system.[3] Signal saturation suggested product inhibition, wherein both the vancomycin and D-Ala–D-Ala fusogens were localized on the fused-vesicle product and incapable of further formation of transvesicular complexes.

Product Inhibition Is Inherent to Synthetic Fusion Systems

The anticipated vesicle composition from one round of fusion between neutral 1% Kaa LUVs and 10% POPG LUVs is 5% POPG and 0.5% Kaa. On the basis of the charge-dependent partitioning coefficient of the magainin surface

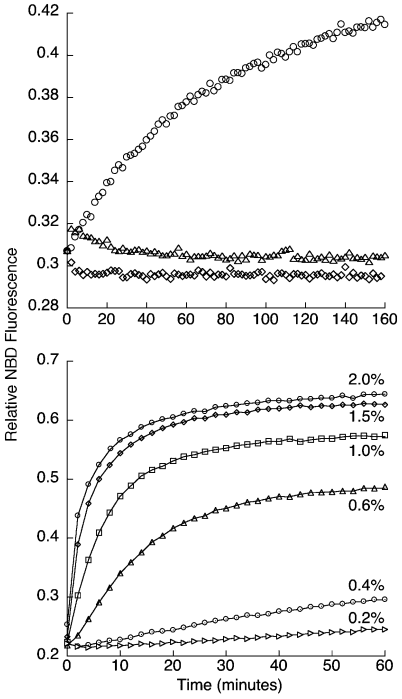

FIGURE 2. (top) Lipid mixing (fusion) driven by vancomycin fusogens (○) that is suppressed by replacement of vancomycin–magainin with magainin alone (△) or by free vancomycin (◇). (bottom) Lipid mixing as a function of D-Ala–D-Ala lipid fusogen mole fraction.[13,14]

anchor, it was expected that the MV conjugate should primarily be bound to the fusion product by both Kaa and electrostatic interactions. We tested this notion by examination of a "completed" fusion reaction in which both fluorescence and size change had reached a steady state (Figure 3). The reaction mixture, in which the fusogens were putatively colocalized on the same 5% POPG product vesicle population, was treated with an unlabeled vesicle preparation bearing 20% POPG. It was expected that the higher negative charge on the 20% POPG surface would competitively bind the VM fusogen, permitting the formation of new trans complexes between 20% POPG vesicles and the 5% POPG fusion product. Indeed, additional rounds of fusion were observed when the system was treated with vesicles with higher negative surface charge. Thus, product inhibition predictably occurs as the charge gradient between the reactant and product vesicles erodes, reinforcing our model of the fusion process in this system. Fusion is essentially unidirectional, mirroring viral membrane fusion processes; both are distinct from synaptic vesicle fusion machinery, which maybe "reset" via active ATP-driven processes.[3]

Contents Loss during MV–Kaa Fusion of Synthetic Vesicles

Synaptic vesicles, which are charged with the task of delivering small-molecule neurotransmitters to the synapse via membrane

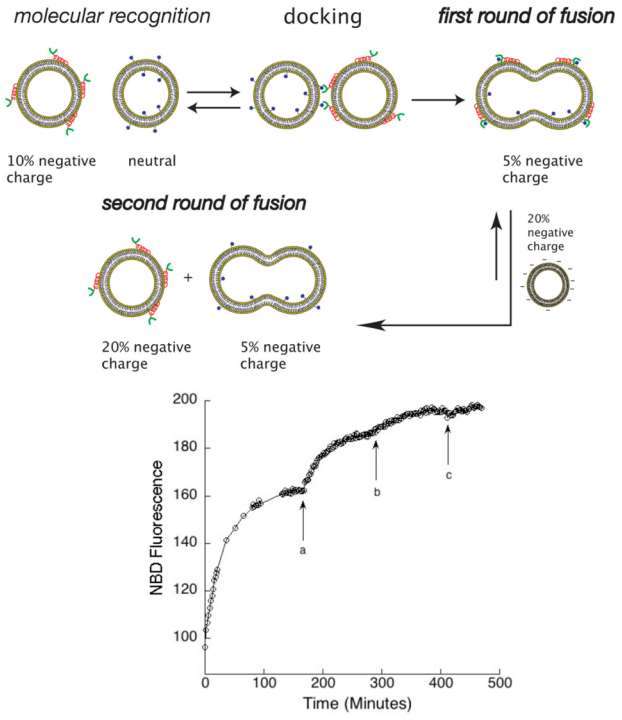

FIGURE 3. (top) Charge gradients overcome product inhibition. (bottom) Multiple rounds of fusion induced by addition of LUVs with higher POPG content (20%) than the starting system (10%) at the time points indicated by a, b, and c.

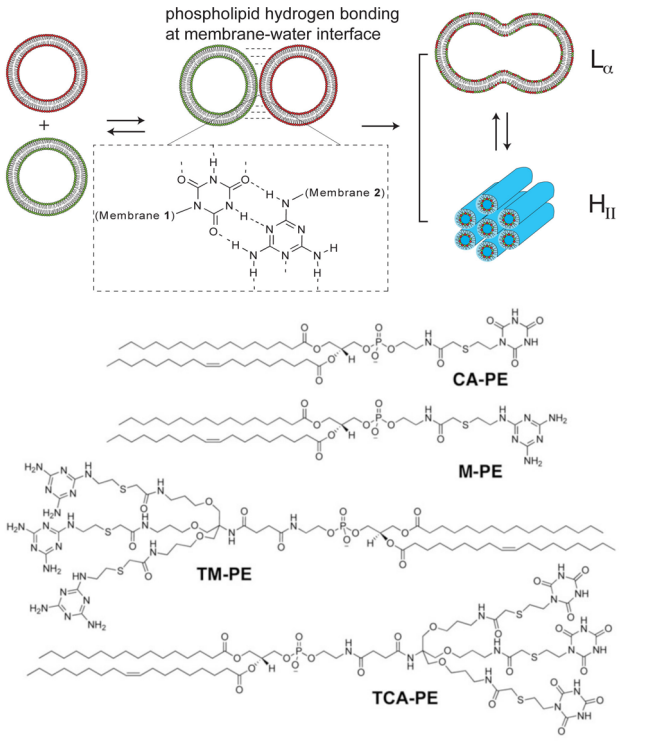

FIGURE 4. (top) Illustration of fusion driven by hydrogen-bonding recognition between (left) CA and (right) M. (bottom) Structures of "monovalent" CA-PE and M-PE and "trivalent" TCA-PE and TM-PE.

fusion, must fuse with high-fidelity contents transfer.[17] It is unclear whether this level of control is necessary for virus–host membrane fusion, which requires only that fusion uncoat large macromolecular assemblies into the cytoplasm. Interactions of MV with Kaa phospholipid fuse lipid bilayer vesicles, but loss of encapsulated small molecules occurs,[14] as judged using a contents-release assay in which vesicles encapsulating a self-quenching dye (calcein, carboxyfluorescein) were subjected to the synthetic fusion conditions.[18] In all cases, the encapsulated dye was rapidly dequenched by dilution into the extravesicular space, indicating full contents loss during the fusion reaction. This result is in line with the known lytic function of antimicrobial peptides such as magainin,[15] which led us to consider alternative membrane activation methods.

Membrane Fusion via Recognition of Hydrogen-Bonding Lipids

Biomembrane curvature strain has been identified as an activating mechanism for fusion.[3,17] Lipid hydrogen bonding[19] is known to result in headgroup dehydration, effectively shrinking the lipid headgroup-to-tail volume ratio[18] and generating negative curvature strain. Hydrogen-bonding lipids such as ceramide render membranes unstable with regard to spontaneous fusion, aggregation, and precipitation.[19,20] Additives such as poly(ethylene glycol) (PEG) are known to induce vesicle fusion with contents mixing via surface dehydration;[21] interestingly, lipid-anchored PEG and glycolipids also sterically prevent fusion.[22,23] This literature prompted us to explore lipid hydrogen bonding as a means of both membrane activation and docking. Considerable work on hydrogen-bonded assemblies in organic solvents has been reported,[24] but fewer aqueous-phase systems have been studied. Prior work on hydrogen-bond-directed assembly of aqueous-phase amphiphiles[25] and Kunitake's work[26,27] on hydrogen bonding at the air–water and amphiphile–water interfaces provided the basis for this approach.

Cyanuric acid (CA) and melamine (M) are C_3-symmetric triazines that are perfect complements in terms of molecular geometry and hydrogen-bond donor–acceptor patterning, noncovalently polymerizing to form hydrogen-bonded sheets that precipitate from water.[24] We hypothesized that polyvalent cyanuric acid and melamine membrane surfaces could be formed by lipid assembly and could drive selective vesicle fusion via hydrogen-bonding recognition and surface dehydration (Figure 4). Phospholipids were prepared with cyanuric acid and melamine headgroups[28,29] via coupling of bromoacetylated POPE lipid with thiolated cyanuric acid and melamine derivatives. Phospholipids with one

(CA-PE and M-PE) and three (TCA-PE, TM-PE) heterocycles displayed were prepared. The "monovalent" CA-PE and M-PE lipids could be assembled and extruded into stable, monodisperse vesicle populations composed exclusively of CA or M phospholipids. Mixing complementary CA-PE and M-PE vesicles resulted in rapid and vigorously exothermic lipid mixing and fusion.[28] Analysis by cryo-TEM revealed the formation of nonvesicular structures that are suggestive of the formation of an inverse-micelle lipid phase,[19] consistent with lipid membrane dehydration. The hydrogen-bond-directed assembly of this system is robust, even in competitive aqueous solvent. Lehn reported that assembly of similar lipid heterocycle systems are driven by complementary electrostatic interactions;[30] in contrast, we found that the CA and M vesicles associated despite repulsive electrostatics between the LUVs. The CA−M binding interaction in water is most akin to nucleobase stacking[31] and is indeed compatible with nucleobase structures.[32,33] A key difference from nucleobase interactions derives from the p*K*a of cyanuric acid (6.8); cyanurate anion must be protonated for assembly, resulting in an enthalpic penalty at neutral pH.[31]

The "trivalent" headgroup phospholipids that present three cyanuric acid or melamine rings (TCA-PE and TM-PE, respectively) could drive selective vesicle docking and surface deposition onto patterned supported lipid bilayers (SLBs) at low mole fraction (0.5−1 mol %) but did not activate the membrane toward fusion;[29] indeed, these trivalent modules could operate solely as recognition modules.[34] Membrane anchoring of trismelamine (TM) with magainin (TMM) restored the selective membrane fusion function with TCA-PE vesicles both in suspension and with SLBs. Docked vesicles could also be fused (with contents loss) by the exogenous addition of magainin peptide (Figure 5), underscoring the need for a membrane-disruptive element in fusion. Interestingly, the lytic activity of magainin was significantly enhanced by selective surface binding of TMM peptide with TCA-PE LUVs,[35] indicating that docking can enhance both fusogenic and lytic processes.

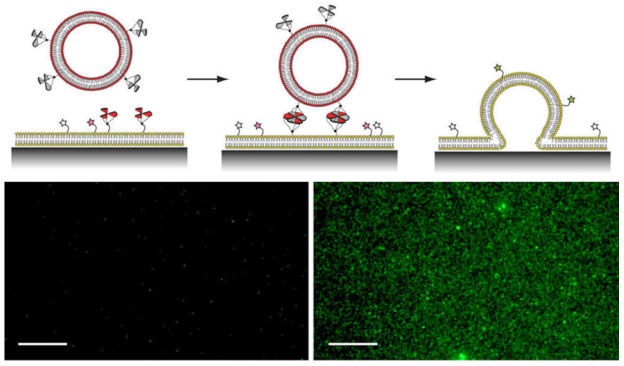

FIGURE 5. (top) Vesicle−SLB fusion followed by dilution of NBD/Rh FRET in the SLB. (bottom) NBD fluorescence of TCA-PE SLBs treated with LUVs and (left) magainin or (right) TM-PE and magainin.[29]

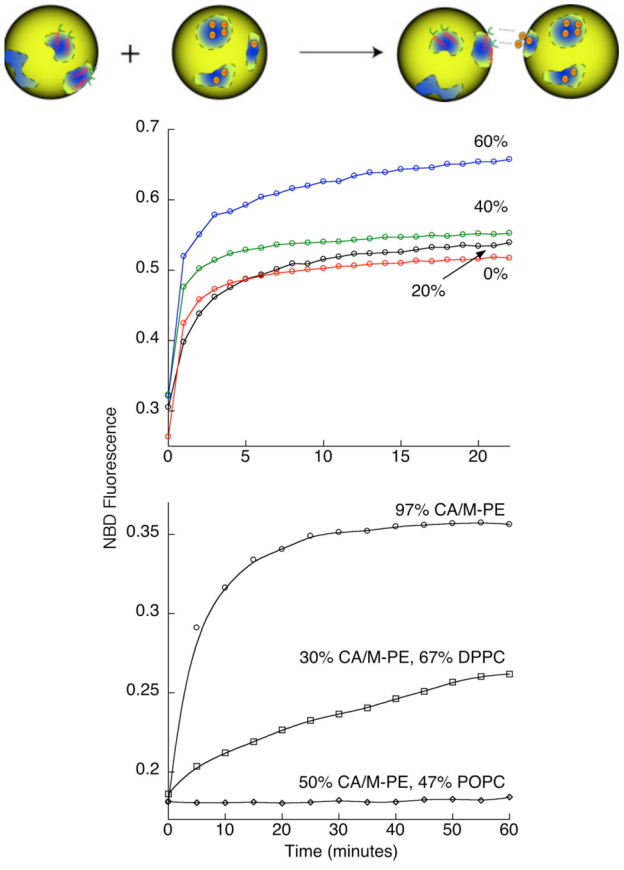

FIGURE 6. (top) Schematic of fusogen clustering into subdomains. (bottom) Lipid mixing data with NBD/Rh-PE (1.5% each): (upper plot) vancomycin-driven fusion for various proportions of DPPC as shown; (lower plot) CA-PE/M-PE-driven fusion of LUVs with compositions as shown.

Effects of Fusogen Concentration and Lipid Composition

The lipid mixing rate and yield are strongly influenced by the fusogen concentration (as expected) and the lipid composition for both the vancomycin- and CA/M-driven systems. The observation of "leaky fusion" in these fluid-phase lipid membrane systems prompted the investigation of more stable membranes such as DPPC (gel phase at 25 °C) and cholesterol-rich lipid compositions. The expectation was that greater stability would result in a diminished fusion rate but reduced contents loss. Indeed, ePC (fluid phase at 25 °C) and cholesterol membranes reacted more slowly but showed only minor decreases in leakage. However, ePC/DPPC mixtures showed both inhibited leakage and a significant increase in the fusion rate with the increase in mole

fraction of DPPC. A similar observation was made with the CA-PE/M-PE lipids, which are anchored with an unsaturated lipid, POPE. When the hydrogen-bonding lipids were diluted with ePC from 100% to 50%, all of the docking and fusion activity was abolished. In contrast, dilution with the saturated lipid DPPC restored the fusion activity in large part at just 30% CA/M lipid to 68% DPPC. These observations are suggestive of fusogen clustering driven by subdomain formation in binary lipid mixtures of saturated and unsaturated phospholipids (Figure 6). Such phase separation has been experimentally observed in ternary mixtures including cholesterol,[36] though there is no direct evidence of lateral lipid organization in more complex lipid compositions containing surface reactive groups.

Macromolecular Fusion Systems and Contents Mixing

The driving forces for CA/M aqueous-phase recognition are similar to those involved in DNA base pairing in that the assembly derives from highly exothermic "base stacking"[37] of cyanuric acid and melamine rings. DNA duplex formation is more directly analogous to the macromolecular fusion motifs found in native systems and serves as an effective fusogenic trigger, as do PNA interactions.[38] Höök and co-workers reported that sterol-anchored 24-mer oligonucleotides that are partially duplexed[39] mediate the fusion of complementary lipid vesicle populations via a DNA-duplex exchange process that "zippers" the membranes together by gathering the membrane anchors at one end of a macromolecular bundle formed across a vesicle–vesicle interface. Much like the model proposed for SNAREpin fusion,[3] this process may activate the membrane via physical strain and exclusion of water from the intervesicular space by macromolecular interactions. Boxer and co-workers devised a similar approach using glyceroldiether phosphoramidites to terminate oligonucleotides, thus installing two-chain membrane anchors[40] that allowed single-stranded oligonucleotides to be used as fusogenic motifs (Figure 7). The anchors must terminate the DNA in such a way as to contract vesicle apposition upon formation of an antiparallel DNA duplex in trans: complementary strands both anchored at the 3′ end or both anchored at the 5′ end yield docked vesicles separated nominally by the length of the antiparallel DNA duplex. Anchoring one oligonucleotide population at the 3′ end and the other at the 5′ end couples the duplex formation and draws the termini and the membrane surfaces closer. Notably, Boxer and co-workers found that in addition to full membrane fusion, contents mixing was also observed.[41]

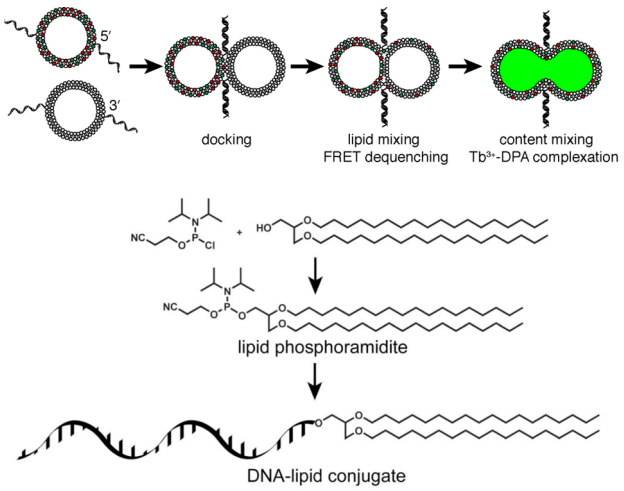

FIGURE 7. (top) Illustration of DNA-duplex-driven vesicle fusion with contents mixing. (bottom) DNA lipidation with glyceroldiethers.

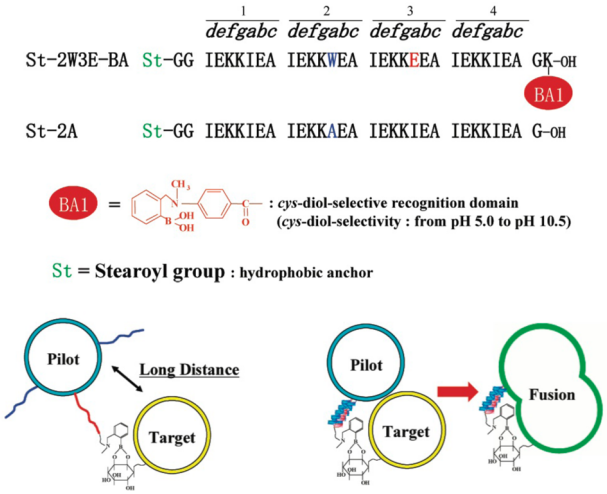

FIGURE 8. (top) Acidic coiled-coil trimer sequence and sugar-targeting boronate lipid. (bottom) Illustration of boronate–sugar vesicle docking followed by acid-triggered peptide folding and apposition, leading to fusion.[46]

Non-SNARE peptide coiled coils have also been studied as fusogenic recognition triggers with native[42] and non-native SNARE anchors.[43,44] Kashiwada, Tanaka, and co-workers developed an elegant system modeled conceptually on the acid-triggered membrane activity of the influenza fusion peptide (Figure 8).[43,45,46] Glutamic acid-rich peptide sequences were membrane-anchored by stearylation of a lysine side chain. The sequences were programmed to fold into parallel coiled-coil trimers by virtue of an isoleucine hydrophobic core.[47] At neutral pH, assembly was inhibited by ionization of the glutamic acid side chains; acidification resulted in nondirectional vesicle assembly and fusion, yielding a pH-controlled fusion event. The authors also utilized a synthetic boronate lipid to selectively direct fusion

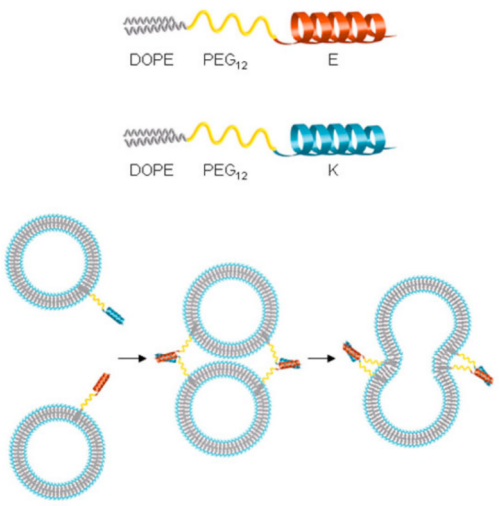

FIGURE 9. (top) Architecture of coiled-coil Velcro dimer fusogens, with acidic (E) and basic (K) sequences indicated. (bottom) Illustration of dimer formation in trans, driving selective vesicle fusion.[44]

with phosphoinositol lipid vesicles.[45] Interestingly, the linker chemistry between the recognition and anchoring groups was critical, as reported by others. These systems exhibited contents mixing as well as lipid mixing, with fusion rates ranging from days to minutes depending on the configuration. Kashiwada and co-workers greatly improved the fusion efficiency by combining the boronate and the HA peptide in a single fusogen.[45] Nonfusogenic vesicle docking could be accomplished by binding of boronate to PI lipids at pH 7.3; acidification to pH 4.3 then triggered fusion. Kros and co-workers also reported the use of coiled-coil dimer-forming peptides as a fusion triggers (Figure 9).[44,48] Rather than using pH-triggered peptide homotrimerization, the authors employed a "Velcro" heterodimer design, anchoring strongly basic and acidic peptides with dimer-forming isoleucine–leucine cores in two different vesicle populations. Intramembrane homodimerization was disfavored by electrostatic repulsion, thus allowing vesicle docking to occur via peptide heterodimer formation. Like Kashiwada and co-workers, they used an oligoethylene linker to connect the helical peptide domain to the phospholipid (DOPE) anchor, and vesicle fusion (20%) was accompanied by 10–15% contents mixing over the same time period, similar to the DNA systems. Thus, it appears that contents transfer is possible in synthetic selective vesicle fusion systems, though the design principles that govern this activity are not clear.

Fusion and Lytic Membrane Activity in HIV Fusion Peptide Variants

We hypothesized that a general peptide solution for exclusive and full fusogenic activity could be derived from analysis of native viral fusion peptides.[4] Using the native HIV fusion peptide sequence as a starting point, we synthesized 38 fusion peptide variants and evaluated their fusion and lysis activity.[49] From this limited libary, we identified many non-native sequences with fusogenic activity similar to or higher than that of the native HIV peptide but with greatly suppressed or nonexistent lytic activity relative to the native sequence, which exhibits high fusogenic and lytic activity. The fusion peptide is an N-terminal 23-residue domain of the fusogenic surface glycoprotein gp41 that is essential to viral infectivity.[4] Like the fusion peptide from influenza virus, the HIV fusion sequence has high glycine content; mutation of these glycines drastically reduces its infectivity.[50] Seemingly contradictory correlations of fusogenicity with α-helical, β-sheet, or indeterminate fusion peptide secondary structure have been proposed.[5] Fusogenic function might be imparted at the level of general physical properties without particular folded-state requirements, as has been found for antimicrobial peptides;[1] this would mean that there are many possible fusogenic sequences. Indeed, Langosch has reported that de novo peptides based on transmembrane domains[9] induce membrane fusion. Using our limited library of HIV fusion peptide variants, we sought to identify determinants of fusogenic function within the framework of the HIV fusion peptide so that one might enhance fusogenic function and minimize membrane permeabilization. Each library member was composed of a 23-residue hydrophobic domain coupled to a hexaarginine–tryptophan (R_6W) C-terminal sequence to enable solubility and quantitation. This diblock amphiphile architecture has been used in fusion peptide studies by Weliky[51] and others.[5] We studied the correlation of membrane activity with glycine content, hydrophobicity, and flexibility within this library. To interrogate the functional importance of the glycine patterning, we "shuffled" the six glycine residues into 11 non-native patterns within the 23-residue HIV fusion peptide sequence (Table 1). This had a significant effect on the fusion/lysis activity, but the systems remained membrane-active, supporting the notion that there are many possible peptide fusogens within the framework of the HIV fusion peptide composition. Further synthesis and evaluation indicated that glycine is not strictly required at all and that the membrane activity survives broad sequence variation as long as the calculated peptide hydrophobicity remains within a functional window (Figure 10). Notably, we were successful in identifying many peptides (including a glycine-free peptide) with high fusogenic activity but minimal lytic function, as judged by lipid mixing, dye release, and giant unilamellar vesicle (GUV) fusion experiments. Confocal fluorescence microscopy imaging of a mixture of two GUV populations with green and red

TABLE 1. Activity of Selected HIV Fusion Peptide Variants

#	Native fusion peptide sequence	fusion %	lysis %
1	AVGIGALFLGFLGAAGSTMGARS-R_6W	69	57
	Glycine Shuffled		
2	GAVIGALGFLFGLAAGSTMGARS-R_6W	71	15
3	AVIGALFGLFLGAASGTMAGRSG-R_6W	66	74
4	AVIALFLGGGFLAASGGGTMARS-R_6W	63	12
5	AVIGGALFLGGGFLGAASTMARS-R_6W	55	25
6	AVIGALFGLFGGLAASGTMGARS-R_6W	55	55
7	AVIGGGALFLGGFLAASGTMARS-R_6W	50	21
8	AVIGGALFLGGFLGAASGTMARS-R_6W	46	25
9	AVIAGGLFLFGGLAASGGTMARS-R_6W	43	33
10	AVIGGALFLGFLGAASGGTMARS-R_6W	39	19
11	AVIALFLGGGGGGFLAASTMARS-R_6W	36	35
12	AVIGGALFLGGFLAASGGTMARS-R_6W	33	16
	Glycine Replacement		
31	AVAAAAVAVAAAVAAAAAVAARS-R_6W	73	2

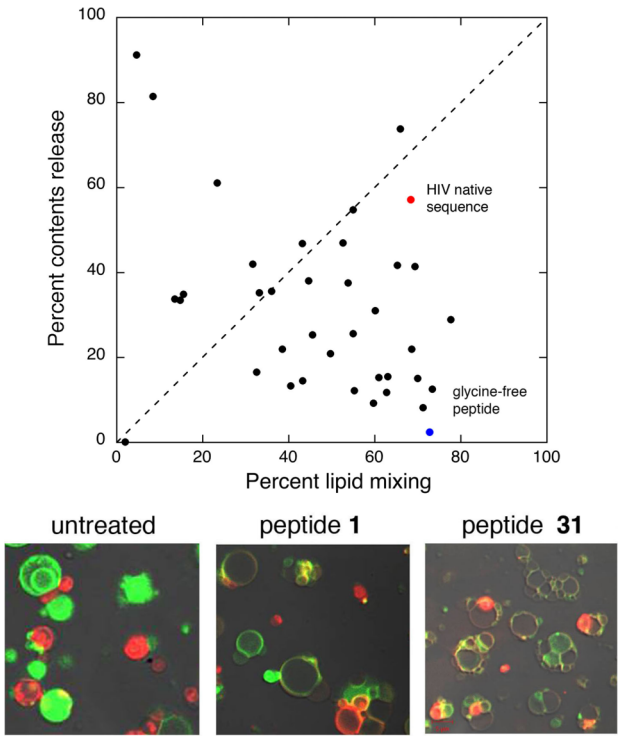

FIGURE 10. (top) Distribution of activity of HIV fusion peptide variants, with the native sequence shown in red and the glycine-free peptide 31 shown in blue. (bottom) Fusion of red- and green-fluorescence-labeled GUVs with the native sequence and glycine-free peptide 31.

fluorescence labels indicated extensive colocalization of the fluorescence following treatment with both the native HIV fusion peptide and glycine-free variant 31 (Figure 10). The calculated hydrophobicity of 31 would not be expected to impart activity unless a helically folded structure is assumed. Indeed, strongly fusogenic peptide variants exhibited helical circular dichroism signatures in SDS–peptide micelles, suggesting a functional connection to secondary structure: peptides that fold on partitioning exhibit stronger membrane binding due to burial of the polar amide backbone via intramolecular hydrogen bonding.[5] Thus, it was possible to identify nonlytic, glycine-free fusogenic peptides with reduced hydrophobicity relative to the HIV fusion peptide. Such peptides are easier to synthesize and handle. Within the cationic–neutral diblock peptide framework, it appears possible to prepare moderately hydrophobic peptides that fuse negatively charged vesicles with minimal lysis but without selectivity. Synthetic fusion peptides of this type are currently under investigation as nonlytic triggers for fusion of docked vesicular complexes. These concepts may be used to design peptides with membrane transport function that have applications in delivery.[52]

Conclusions

The selected artificial membrane fusion systems discussed here emerge directly from observations made regarding native fusion machinery. Through reductionist chemical approaches and de novo design, membrane fusion function has been made accessible. Furthermore, artificial systems have allowed concepts from biological fusion systems to be generalized and biophysically tested in ways that are not yet possible with living membranes. There remains a considerable divide between the high fusion rate and control observed in native systems relative to designed fusogens, though it is anticipated that further design will yield improvements in efficiency. In particular, the origin of high-fidelity contents transfer during fusion that is observed in some systems has not yet been elucidated. These fundamental issues of membrane transport are uniquely addressable in model membranes by synthesis and design and could find applications in materials design and controlled chemical delivery.

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BIOGRAPHICAL INFORMATION

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Dennis Bong was born in Victoria, British Columbia, in 1974. He obtained a B.Sc. in Chemistry at UC Berkeley while working with Peter Vollhardt. He obtained his Ph.D. in 2001 at the Scripps Research Institute under the direction of Reza Ghadiri, followed by a postdoctoral stint at Columbia University with Ronald Breslow. His research group focuses on the design and application of functional molecular recognition in aqueous-phase assembly systems.

FOOTNOTES

The authors declare no competing financial interest.

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