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# Lipid Membrane Adhesion and Fusion Driven by Designed, Minimally Multivalent Hydrogen-Bonding Lipids

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***Abstract:*** Cyanuric acid (CA) and melamine (M) functionalized lipids can form membranes that exhibit robust hydrogen-bond driven surface recognition in water, facilitated by multivalent surface clustering of recognition groups and variable hydration at the lipid−water interface. Here we describe a minimal lipid recognition cluster: three CA or M recognition groups are forced into proximity by covalent attachment to a single lipid headgroup. This trivalent lipid system guides recognition at the lipid−water interface using cyanurate−melamine hydrogen bonding when incorporated at 0.1−5 mol percent in fluid phospholipid membranes, inducing both vesicle−vesicle binding and membrane fusion. Fusion was accelerated when the antimicrobial peptide magainin was used to anchor trivalent recognition, or when added exogenously to a preassembled lipid vesicle complex, underscoring the importance of coupling recognition with membrane disruption in membrane fusion. Membrane apposition and fusion were studied in vesicle suspensions using light scattering, FRET assays for lipid mixing, surface plasmon resonance, and cryo-electron microscopy. Recognition was found to be highly spatially selective as judged by vesicular adhesion to surface patterned supported lipid bilayers (SLBs). Fusion to SLBs was also readily observed by fluorescence microscopy. Together, these studies indicate effective and functional recognition of trivalent phospholipids, despite low mole percentage concentration, solvent competition for hydrogen bond donor/acceptor sites, and simplicity of structure. This novel designed molecular recognition motif may be useful for directing aqueous-phase assembly and biomolecular interactions.

## Introduction

Selective aqueous phase hydrogen bond-driven molecular recognition faces the challenge of solvent competition. While there are many solutions to this problem in Nature, there are few non-native designed systems known.[1−8] We have previously reported our aqueous-phase studies on cyanurate (CA) and melamine (M) phospholipid derivatives that drive membrane chemistry via hydrogen-bonding interactions between the CA and M headgroups.[9] There have been numerous studies on the CA-M system; though the parent compounds readily cocrystallize in a hydrogen-bonding network in water[10−12] and derivatives are well-known to assemble in low dielectric solvents[13−16] or when hydrophobically buried,[17] derivative assembly generally does not occur in hydrogen-bonding solvents.[12,18−20] Indeed, without the phospholipid module, monoderivatived cyanurate (CA) and melamine (M) neither assemble nor inhibit interactions between the lipids in water (Figure 1).[9] Surface multivalency of an assembled membrane provides binding avidity that greatly enhances the hydrogen-bonding interaction at the lipid−water interface.[17,21−23] The requisite density of CA/M modules for recognition was indicated

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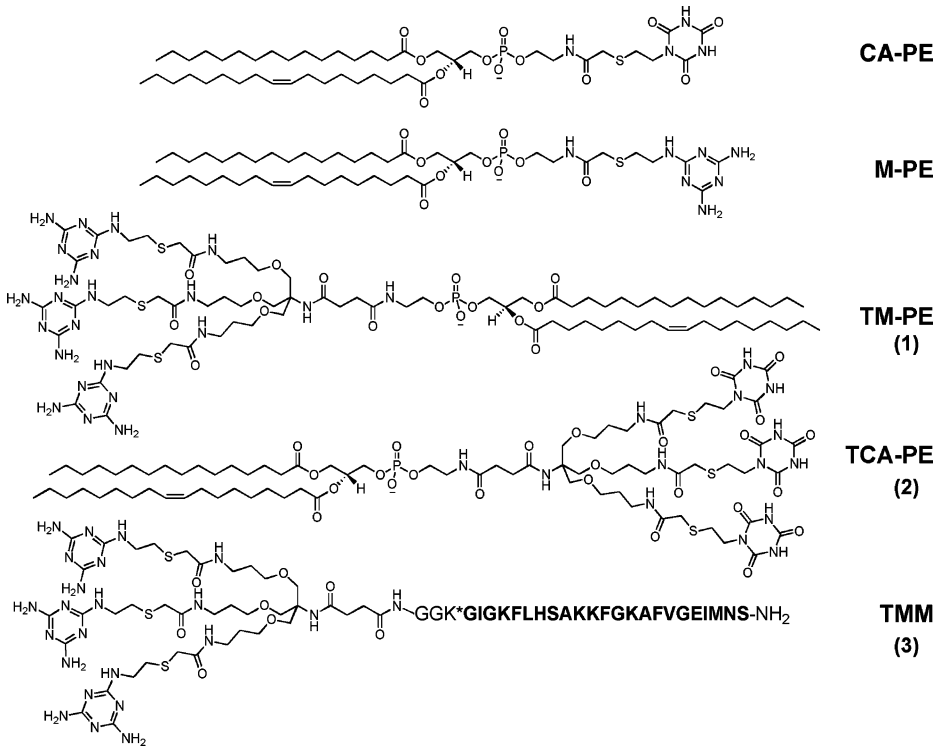


***Figure 1.*** Structures of compounds used in this study (CA-PE and M-PE have been previously reported). For TMM, the sequence of magainin is shown in bold and K* is lysine(acetamidobenzamide).

by diminished interaction at CA-PE/M-PE concentrations of less than 70% in fluid phase (ePC) membranes. Notably, when CA-PE and M-PE were diluted in membranes with gel-phase phospholipids (DPPC), 30% of CA/M lipid was sufficient for vesicle binding,[9,24] presumably due to clustering of CA/M headgroups upon phase separation of CA/M-PE lipids from the gel-phase lipids.[25,26] These findings prompted our investigation of the minimal valency required for detectable lipid−lipid binding. This exploration was guided by the notion that each lipid can only interact with its surrounding neighbors at any given moment; thus, recognition must be facilitated by nearest neighbors. Separation of hydrogen-bonding (HB) groups by just one lipid (50 mol% concentration in ePC) abrogates lipid−lipid binding, implying that clustering of HB groups is essential.[9] We synthesized lipids in which three CA or M groups were forced into proximity by covalent attachment to the same phospholipid and found that this minimal design imparts robust molecular recognition and function at the lipid−water interface (Figure 2).

## Results and Discussion

**Design and Synthesis.** *Tris*(hydroxymethyl)aminomethane was readily functionalized to symmetrically install three terminal chlorides and one terminal carboxylate, compound **6** (Scheme 1). Amide coupling of the carboxylate to 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine lipid (POPE) yielded a trichloride lipid that was easily transformed to trivalent melamine (TM-PE, **1**) or cyanurate (TCA-PE, **2**) lipids via thioether formation, with three hydrogen bonding heterocycles per lipid headgroup (Figure 1, Scheme 1). These lipids were incorporated into synthetic lipid membranes, and their ability to guide surface recognition and fusion was evaluated. In addition, the trichloride headgroup (**6**) was coupled to the N-terminus of the membrane-active peptide magainin;[27−29] thioether formation with melamine-thiol yielded a peptide conjugate (TMM, **3**) that combines both molecular recognition (TM) and membrane activation, in analogy to previously reported systems[24,30] (Scheme 1). We investigated intermembrane lipid recognition in three different contexts: (1) lipid−lipid binding (TCA-PE/TM-PE), (2) lipid−lipid binding with membrane activation by magainin (TCA-PE/TM-PE + mag), and (3) lipid−peptide binding (TCA-PE/TMM). These systems were designed to explore the nature of TCA/TM headgroup binding and the extent of membrane fusion with and without a known disruptive element such as an antimicrobial peptide.

**Vesicle−Vesicle Binding.** Lipid films were prepared that contained TCA-PE at 0.1−5 mol% in egg phosphatidylcholine (ePC) and TM-PE at the same concentration in 20% phosphatidylglycerol lipid (POPG) and ePC. TM-PE liposomes have a propensity for self-aggregation that is completely suppressed by the inclusion of negatively charged POPG in the preparation. These lipid films were hydrated in phosphate buffer saline (PBS) at pH 6.7 or 7.4 and extruded through 100 nm pore polycarbonate membranes to produce large unilamellar vesicles (LUVs) that appeared to be monodisperse and nonaggregated, as judged by dynamic light scattering (DLS) and cryo-electron microscopy (cryo-EM). While electrostatically driven binding between oppositely charged membranes and macromolecules in water

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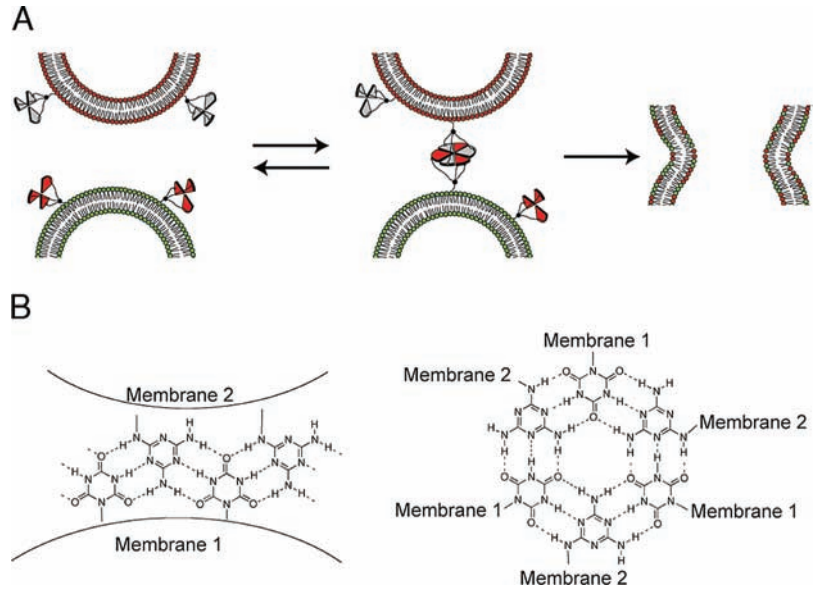
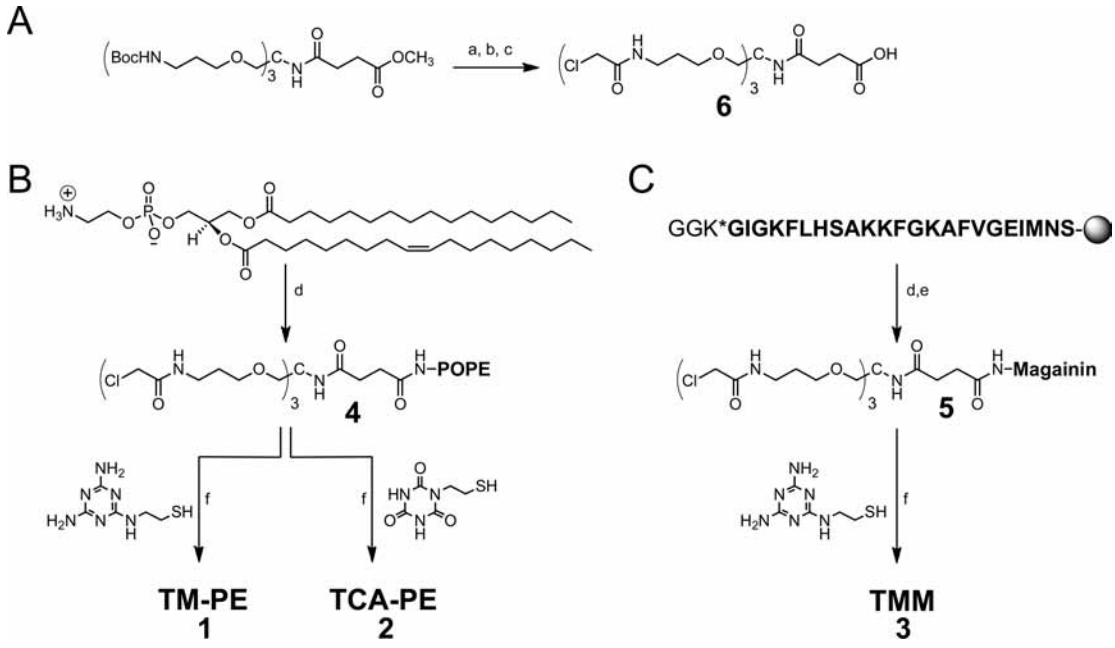



***Figure 2.*** (A) Schematic illustration of how membrane anchored lipids trifunctionalized with CA or M (symbolized as red and gray wedges) might direct lipid membrane apposition and fusion. (B) Possible modes of intermembrane hydrogen bonding, based on known CA/M assembly topologies: tape (left) and rosette (right). The actual hydrogen-bonded structures formed have not been established.

***Scheme 1***




(a) TFA, $H_2O$, $CH_2Cl_2$; (b) chloroacetic anhydride, DIEA, $CH_2Cl_2$; (c) LiOH, MeOH; (d) **4**, HBTU, DIEA, DMF; (e) 95% TFA, 5% $H_2O$ and (F) DMF, $CHCl_3$, DIEA.

is well-established,[31] we observe a phenomenon upon mixing of TCA-PE and TM-PE membranes consistent with molecular recognition between neutral components. Notably, despite repulsive potentials of −13 and −23 mV for the TCA-PE and TM-PE LUVs, respectively, mixing the vesicle populations in a 1:1 ratio resulted in rapid doubling of size as judged by DLS (Figure 3). At pH ≈ 7, the cyanuric acid and melamine groups should be neutral, thus the charge on TCA-PE and TM-PE should be −1 due to the phosphate. Replacing TCA-PE and TM-PE with the singly charged POPG yields similar LUV ζ potentials (−11 and −23 mV, respectively) suggesting that the charge on each lipid is approximately −1, as expected. Additionally, increasing salt concentration slightly enhances vesicle−vesicle aggregation, consistent with a neutral rather than electrostatic interaction.

We probed this interaction further by surface plasmon resonance (SPR) experiments in which one of the LUV populations was surface bound on an SPR substrate while the complementary LUVs were flowed over the modified surface (Figure 4). While control LUVs resulted in insignificant changes in refractive units, complementary LUVs produced strong, concentration-dependent signals consistent with surface deposi-

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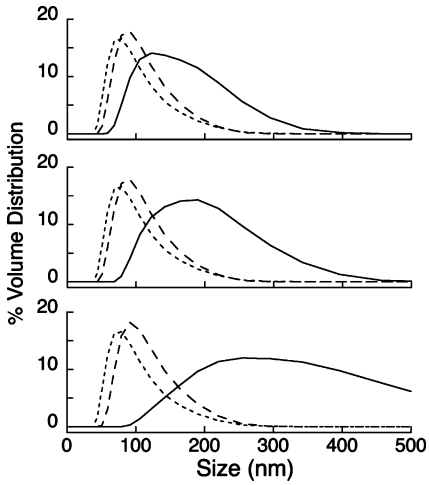


***Figure 3.*** Size change of vesicles as a function of vesicle aggregation or fusion, measured by DLS. (Top) TCA-PE LUVs reacted with TM-PE LUVs; (Middle) Same as top, in presence of magainin; (Bottom) TCA-PE LUVs reacted with TMM bound to POPG LUVs. Traces represent: TCA-PE (---); TM-PE and TMM/POPG (− −); mixed LUVs after 30 min equilibration (—).

tion (Figure 4A); this behavior was similar regardless of which lipid was on the surface and which was in suspension. Binding dropped off sharply when TM-PE LUV concentrations below 1% but was still detectable at 0.1% (TCA-PE constant at 0.3% in surface-bound LUVs, Figure 4B). Interestingly, layer-by-layer deposition was not observed. This suggests that the TCA-PE and TM-PE lipids become concentrated at the interface between the apposing membranes, leaving only nonbinding phosphatidylcholine exposed. Furthermore, concentration changes only affected on-rates, with no apparent desorption observed even at the lower limit of detection, consistent with an increase in multivalency/binding avidity[32] following initial docking through migration of recognition lipids to the LUV−LUV interface. Undetectable off-rates could also arise from noncovalent reaction. We examined the possibility of membrane fusion catalyzed by low concentrations of TCA/TM recognition using standard FRET dilution assays for lipid mixing.[47]

**Vesicle−Vesicle Fusion.** Synthetic vesicle fusion systems driven by molecular recognition between coiled-coil peptides[33,34] and proteins derived from the SNARE synaptic vesicle fusion machinery,[35−38] nucleic acid,[39−41] and small molecules[9,24,30] have been previously reported, as well as systems driven by metal complexation[42−44] and electrostatics.[31,45] We have found that the designed aqueous-phase interaction between TCA/TM-PE lipids can also drive selective membrane merger, though this occurs with contents leakage.[46] Lipid mixing was taken to represent membrane fusion and was followed by monitoring loss of FRET between NBD-PE and Rh-DHPE lipids in an acceptor vesicle upon fusion with an unlabeled vesicle.[47] We examined three distinct topologies for membrane reactions mediated by TCA and TM: (1) intermembrane lipid−lipid binding, (2) intermembrane lipid−lipid binding with membrane-binding peptide added as a third component, and (3) intermembrane lipid−peptide binding. Though mixing the TCA-PE and TM-PE LUVs resulted in aggregation (Figure 3), no lipid mixing was observed, even when a large excess of the nonlabeled vesicle was used (Figure S1 of the Supporting Information), suggesting that membrane fusion is arrested in the docking stage (Figure 5A). Addition of magainin peptide to the docked system triggered rapid lipid mixing, indicating that disruptive peptide-membrane binding may facilitate membrane fusion. The identical experiment was carried out with TMM (Figure 1) replacing TM-PE and magainin; reaction of these vesicles with 5% TCA-PE LUVs also resulted in efficient lipid mixing. Fusion with TMM was concentration dependent, with a minimum surface concentration of 2% required when the reacting membrane contained TCA-PE at 2% (Figure 5B). Introduction of the TCA or TM headgroup alone (without membrane anchor) to the TMM fusion system resulted in significant inhibition of lipid mixing, suggesting that the soluble trivalent headgroups were capable of blocking surface binding sites and suppressing membrane apposition (Figure 5C). Interestingly, inhibition with TM was more effective than with TCA, though both elicited a decrease in lipid mixing. While the origin of this difference is unclear, the possibility of selective molecular recognition between designed small molecules in aqueous milieu is intriguing; we are investigating these interactions further. We previously reported that LUVs of 100% M-PE and CA-PE fused efficiently, so we compared the efficiency of fusion when the 100% M-PE LUVs were replaced with 5% TM-PE in a POPG membrane. Surface dehydration by hydrogen bonding has not yet been identified as a major mechanism for native membrane fusion, but appears to be an effective strategy that is enhanced when combined with membrane disruption by a peptide anchor. Fusion was observed when 5% TM-PE LUVs replaced 100% M-PE LUVs, but both reactions were markedly accelerated by addition of magainin peptide as catalyst, yielding similar lipid mixing rates (Figure 5D). These findings are consistent with the notion that extensive dehydration is necessary for fusogenic activation via surface H-bonding; TM-PE/CA-PE binding likely results in a smaller contact area and thus requires peptide catalyst for productive docking. With regard to recognition, the similar fusion rates produced by 5% TM-PE and 100% M-PE in the presence of magainin indicate that the covalent cluster of three

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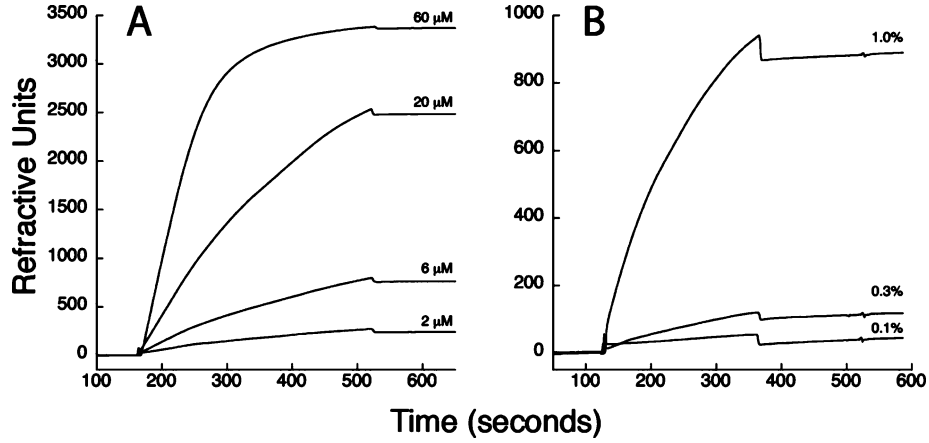


***Figure 4.*** Concentration dependent SPR traces of complementary LUV interactions. (A) Total lipid concentration is shown; trivalent lipids are at 5% of total (ePC and POPG) in both surface bound and suspension LUVs. TM-PE LUVs flowed over chip with TCA-PE LUVs bound. (B) Total lipid concentration in suspension was maintained at 60 $\mu$M with mol% of TM-PE varied as labeled. Surface bound LUVs had 0.3% TCA-PE for each run.

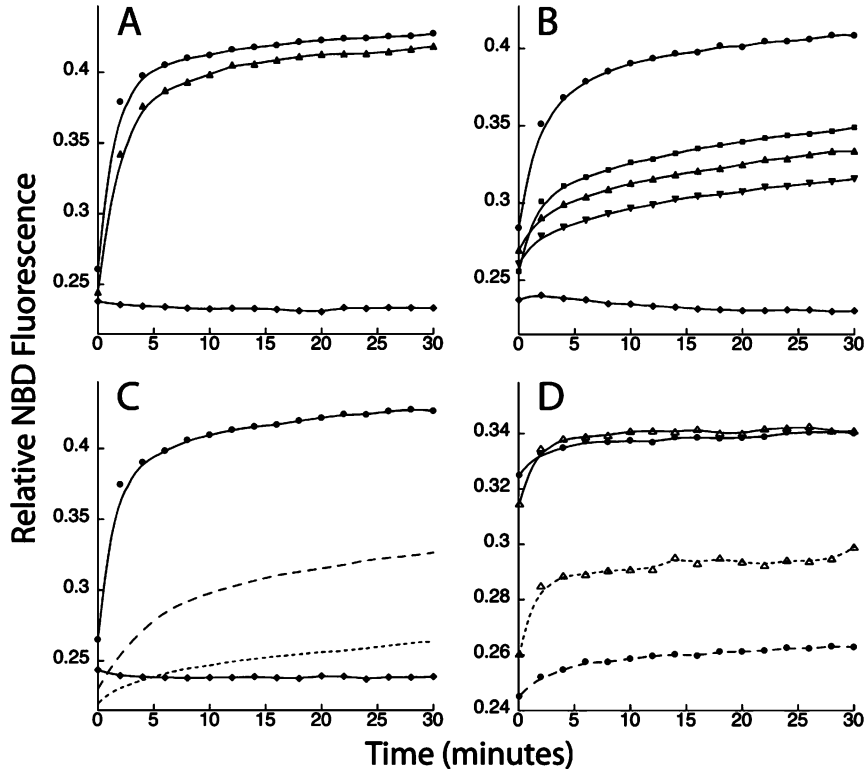


***Figure 5.*** Lipid mixing assay for membrane fusion.[47] NBD-PE and Rh-HDPE were incorporated at 1.5% in the TCA-PE containing LUV, TM, and TCA represent soluble headgroup only, without phospholipid. (A) 3 systems: 5% TMM/POPG/ePC LUVs reacted with 5%TCA-PE/ePC LUVs (●), 5% TM-PE/POPG/ePC LUVs reacted with 5% TCA-PE/ePC and 5% magainin (▲), 5% TM-PE/POPG/ePC reacted with 5% TCA-PE/ePC (◆); (B) concentration dependence: POPG/ePc reacted with 2% TCA-PE/ePC LUVs at 1% (◆), 2% (▼), 3% (▲), 4% (■), and 5% (●) TMM; (C) inhibition: 3% TMM/POPG/ePC LUVs reacted with 3% TCA-PE/ePC LUVs (●), inhibited with 5 equiv. TCA (− −), inhibited with 5 equivalents TM (---), POPG/ePC LUVs reacted with 3% TCA-PE/ePC LUVs with 3% magainin (◆); (D) trivalent/monovalent comparison: 100% M-PE LUVs and 97% CA-PE LUVs with 5% magainin (△) and same, without magainin (-△-), 5% TM-PE in POPG/ePC LUVs reacted with 97% CA-PE LUVs with 5% magainin (●) and same, without magainin (-●-).

melamine rings in TM-PE can produce similar docking effects as an entire surface of M-PE.

**Cryo-Electron Microscopy.** Hydrogen bonding lipids are known to induce lamellar to hexagonal phase transitions,[48,49] as we found with CA/M-PE LUVs.[9] Given the low mole percentage of trivalent lipids in the vesicles studied, we anticipated that the behavior of the membrane should more closely approximate a lamellar phase membrane. We used cryo-TEM to examine 5% TM-PE and 5% TCA-PE LUVs for signs of hexagonal phase formation and vesicular adhesion before and after mixing. The reactant LUVs alone appeared to be nonaggregating, monodisperse populations of LUVs (Figure S2 of the Supporting Information), while the images of the LUV mixture consistently indicated lamellar phase LUVs (Figure 6).

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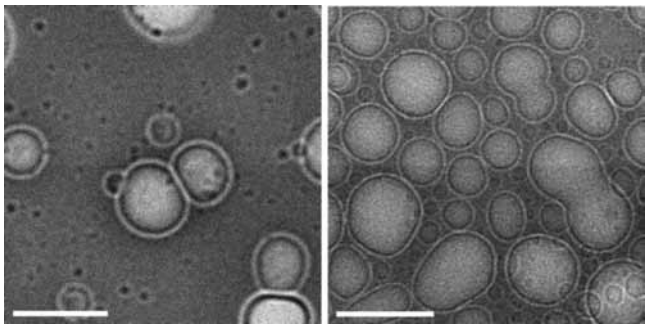

***Figure 6.*** Cryo-TEM of 5% TM-PE/ePC LUVs reacted with 5% TCA-PE/ePC LUVs; (left) adherent vesicles, (right) adherent and possibly fused vesicles. Scale bar = 100 nm.

Vesicular aggregates in the mixture were observed as expected, as well as many structures that appeared to be captured immediately postfusion, with elongated form approximately twice the diameter of a single LUV and a constriction around the center, possibly from the expansion of a fusion stalk. This observation runs counter to the lack of vesicular fusion detected by fluorescence (Figure 5) and suggested that the aggregated vesicles may be metastable with regard to fusion. We postulated that the sharp and extreme temperature decrease inflicted on the sample by plunging into liquid ethane slush during freezing for cryo-EM analysis triggered rapid fusion of the vesicles docked by TCA/TM-PE recognition, resulting in the postfusion vesicle structures. To address this possibility, we formed docked vesicles as previously, with fluorescent lipids to follow lipid mixing, and immersed this sample in liquid nitrogen until frozen. Upon thawing, we found the fluorescence signature of fusion, which was absent in the reactant and unfunctionalized vesicles (Figure S3 of the Supporting Information); multiple freeze−thaw cycles induced fusion in all LUVs, as expected given the cell-membrane rupturing effect of freeze−thaw procedures.

Though this experiment does not duplicate the conditions of cryo sample preparation, the partial fusion resulting from one cycle of freeze−thaw suggests heightened membrane instability in the docked state and supports the notion that cryo-TEM imaging has captured some assemblies shortly after fusion.

**Vesicle Adhesion with Supported Lipid Bilayers.** Supported lipid bilayers (SLBs) are a powerful tool for studying lipid membrane interactions.[50] We constructed SLBs with a 0−5% concentration gradient of TCA-PE across a surface that was patterned with 50 $\mu m^2$ fibronectin grids that act as diffusion corrals.[51] These SLBs were used to examine the ability of TCA/TM-PE recognition to direct spatially selective LUV-SLB deposition on the micrometer scale. Fluorescence microscopy imaging revealed that TM-PE LUV deposition reproduced the surface pattern of TCA-PE with high fidelity, to the extent that adhesion to the unfunctionalized SLB was undetectable (Figure 7). We also monitored deposition of TM-PE LUVs with TCA-PE absent from the bilayer, as well as deposition of simple POPG LUVs onto the TCA-PE SLB; in both cases, no vesicle adhesion was detectable. In fact, adhesion of TM-PE LUVs on the TCA-PE SLB following treatment with POPG LUVs produced an identical result to TM-PE LUV deposition without prior POPG deposition. These data strongly support the notion of spatially selective membrane apposition driven by binding

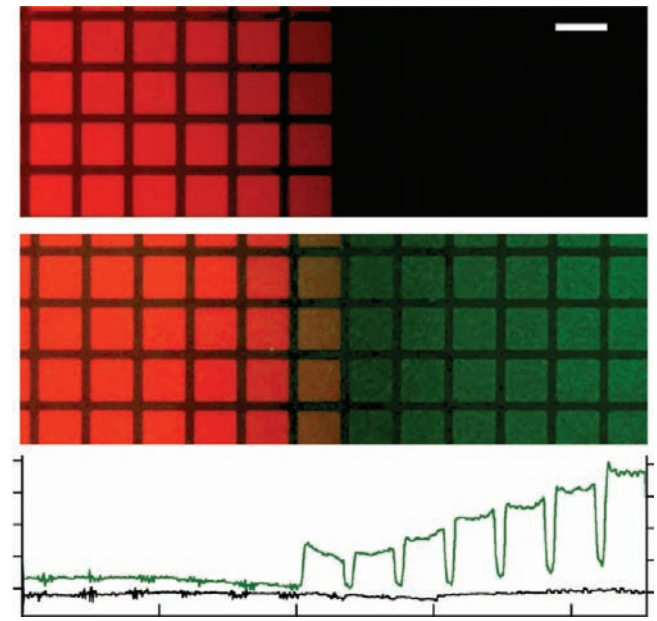

***Figure 7.*** Fluorescence microscopy of a supported lipid bilayer (SLB), formed on a fibronectin grid (scale bar = 50 $\mu$m) with sharp gradients of Texas Red lipid from left to right and TCA-PE from right to left. (Top) Both sides of the SLB were treated with POPG LUVs containing Oregon Green lipid (OG-PE); (Middle) both sides were treated with TM-PE/POPG LUVs containing OG-PE; (Bottom) OG fluorescence across the SLB for top image (black) and middle image (green).

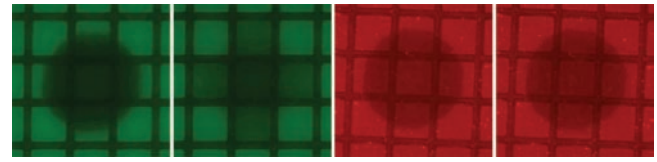

***Figure 8.*** Fluorescence microscopy of a vesicle-bound SLB on a 50 $\mu$m feature fibronectin grid. The SLB was labeled with OG-PE and TCA-PE; vesicles were labeled with TR-PE and TM-PE. Surface was photobleached and FRAP monitored in both the SLB (green) and vesicle (red) layers. (Left) OG-PE fluorescence channel immediately after photobleaching; (Left, Center) 4 min after; (Right, Center) TR-PE channel immediately after photobleaching; (Right) 4 min after.

of TCA-PE and TM-PE. The adhered LUVs (labeled with TR-DHPE) exhibited markedly less lipid mobility than the SLB. While the SLB fluorophore exhibited significant fluorescence recovery after photobleaching (FRAP), the adsorbed layer did not recover after extended monitoring (Figure 8). This differential mobility suggests the absence of SLB-LUV fusion, as expected from vesicle−vesicle fusion experiments (Figure 5) though it is possible that the FRAP experiment is less sensitive to this process. Vesicle fusion with the SLB should lead to similar FRAP in both channels; as it is not observed with vesicle-bound TR-DHPE, then it is likely that TR-DHPE is part of an unfused, multivalently tethered vesicle. The numerous multivalent TCA/TM-PE interactions clustered at the SLB/LUV interface present a significant diffusion barrier, unlike DNA-tethered vesicle systems,[52] possibly due to higher surface concentration. The requirement for FRAP in the adhered LUV layer (assuming no membrane fusion) is essentially desorption/resorption, and our SPR data indicate that this process is extremely slow (Figure 4). Thus, it is likely that the hydrogen

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***Table 1.*** Conditions for LUV−SLB Reaction

| | SLB (in ePC) | LUV (in ePC) | lipid mixing |
|---|---|---|---|
| A | 5% POPG | 25% POPG, 1% TMM | (−) |
| B | 5% TCA-PE | 25% POPG, 1% TMM | (+) |
| C | 5% TCA-PE | 25% POPG, 1% Mag | (−) |
| D | 5% TCA-PE | 20% POPG, 1% Mag, 5% TM-PE | (+) |
| E | 5% POPG | 25% POPG | (−) |
| F | 5% TCA-PE | 20% POPG, 5% TM-PE | (+) |
| G | 5% TCA-PE | 1% TMM alone | (−) |
| H | 5% TCA-PE | 1% Mag alone | (−) |
| I | 5% POPG | 20% POPG, 1% Mag, 5% TM-PE | (−) |
| J | 5% POPG | 25% POPG, 1% Mag | (−) |

bonding lipids involved in the surface contact on both the LUV and SLB are immobile while the fluorescently labeled lipids remain mobile in the continuous SLB.

**Vesicle Fusion with Supported Lipid Bilayers.** LUV−SLB fusion[38] was studied using the same lipid mixing FRET assay as in suspension.[47] Treatment with TM-PE or TMM functionalized LUVs should result in vesicle docking and fusion, depending on conditions (Figure 5). Lipid mixing would dilute the surface bound NBD C6-HPC/Rh-DHPE FRET pair and dequench the FRET donor (NBD); we monitored both NBD and Rh fluorescence channels and evaluated fusogenic conditions using ten experiments (A−J, Table 1). SLB imaging generally tracked with LUV−LUV suspension results. Both TCA/TM recognition groups had to be present to produce a lipid mixing signal while control experiments (A, C, E, G−J, Table 1) yielded minimal signal changes (Figure 9). Replacement of recognition groups with simple charges did not result in detectable reaction, again ruling out electrostatic interactions as the main driver of binding. One notable deviation from LUV−LUV and LUV−SLB experiments (Figures 5 and 7) was the positive fusion signal observed with TM-PE and TCA-PE interactions. Our previous experiments indicated that this system exhibited surface adhesion without fusion and vesicle fusion experiments did not even suggest partial lipid mixing that could be attributed to hemifusion. Under LUV−SLB fusion conditions (Figures 9 and 10F), strong lipid mixing was observed. This again points toward the metastability of the docked system with regard to fusion or hemifusion (these experiments do not distinguish the two), as suggested by cryo-TEM data (Figure 6). Variation in stoichiometry, lipid composition, and fluorophore system between LUV−SLB fusion, LUV−LUV fusion, and LUV−SLB adhesion experiments could cause a range of membrane reactivity and detectable response.[24,40,53] Overall, these data indicate a lipid recognition system in which there is only a slender mechanistic separation of surface recognition and membrane activation and merger, thus minor perturbations in the docked system may spontaneously trigger disruption and lipid mixing.

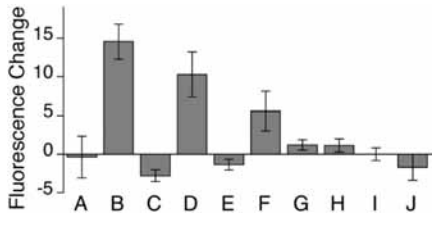


***Figure 9.*** Total integrated NBD fluorescence change on SLB surface after 20 min of reaction and surface washing. Experimental conditions are shown in Table 1. All conditions have 1.5% each NBD-PE and Rh-PE in the SLB. Reactions and measurements were performed in triplicate.

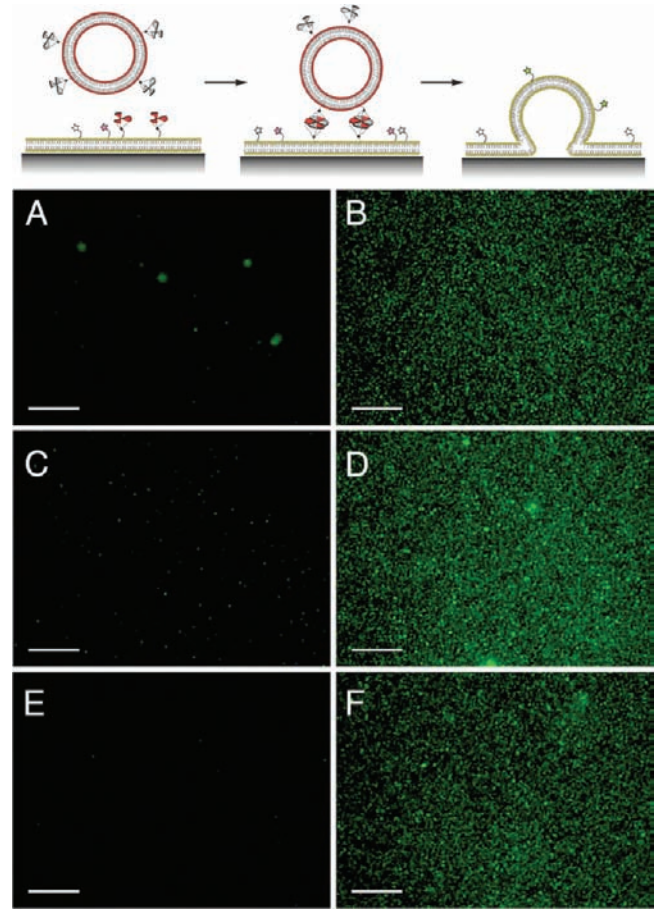


***Figure 10.*** (Top) Schematic illustration of vesicle−SLB fusion, followed by dilution of NBD/Rh FRET in the SLB. (Below) NBD fluorescence microscopy of SLBs containing 2% NBD-C6 HPC, 2%Rh-DHPE in ePC. Images A−F correspond to conditions described in Table 1.

## Conclusions

Our examination of designed lipid recognition at the bilayer−water interface has yielded findings along two fundamental lines: molecular recognition and membrane merger. While selective membrane fusion requires both recognition and disruption, these functions need not be performed by the same molecule. Trivalent lipid−lipid binding can induce membrane apposition, but in the vesicle−vesicle context, no fusion or lipid mixing. Addition of a membrane-disrupting peptide as a third component (TCA-PE + TM-PE + Mag) results in lipid mixing with high efficiency (Figures 5 and 10). Similarly, fusion was observed when one of the components was membrane anchored with the same peptide (TCA-PE + TMM). Given the fusogenicity of the three-component system, it appears that membrane activation need not be precisely at the site of molecular recognition. Though the surface concentration of H-bonding trivalent lipids is relatively low, the docked vesicular aggregate is unstable with regard to fusion, possibly due to headgroup H-bonding which facilitates the formation of nonbilayer fusion intermediates.[48,49] Indeed, the metastability of TCA/TM lipid docked membranes may explain why fusion is not observed in

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LUVs, but observed in LUV−SLB binding and possibly during cryo-TEM sample preparation. Although mechanistic questions remain, these experiments further illustrate the general principles of membrane merger and demonstrate a new designed functional recognition motif.

These studies have revealed that low membrane concentrations of the trivalent CA/M lipids or peptides retain the robust molecular recognition properties found with surfaces composed entirely of monovalent CA/M recognition lipids, though membrane activation upon binding is diminished. The minimal nature of headgroup design and synthetic simplicity stands in contrast to the effectiveness of recognition function. Recognition appears to occur between neutral components: though it is conceivable that a p$K_A$ shift induced at the lipid−water interface could generate a negatively ionized cyanuric acid and a positively charged melamine, we find that the contribution of electrostatic interactions is minor to nonexistent in our membrane-anchored TCA/TM system. Thus, we conclude that the main driving force for recognition is not charge complementation, but rather, a more subtle hydrogen-bond donor−acceptor pattern recognition between largely neutral species in competing aqueous solvent. Moreover, this interaction is sufficiently strong to overcome hydration repulsion forces[54−56] between membranes to induce both spatially selective surface adhesion and membrane merger. The biophysics of this binding interaction in water, as well as the range of aqueous-phase assembly chemistry that may be mediated by cyanurate and melamine groups, remains an intriguing topic with potential relevance to the development of new functional materials.

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**Supporting Information Available:** Detailed synthetic and analytical procedures, compound characterization, additional cryo-EM, and fluorescence data. This material is available free of charge via the Internet at http://pubs.acs.org.

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