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# Membrane Activation: Selective Vesicle Fusion via Small Molecule Recognition

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Modulation of lipid bilayer packing contributes to the activation of biomembrane functions[1] and can initiate *noncovalent* membrane reactions such as lysis, pore-formation, and fusion.[2] While many chemical agents can cause nonspecific lysis and fusion,[3] native membrane chemistry is specific and regulated.[4] The selectivity of membrane fusion is thought to arise from the coupling of membrane perturbation and surface−surface recognition (binding).[4,5] Interestingly, both class I viral and endogenous secretory systems utilize protein coiled-coil recognition as an engine of fusion;[6] indeed, these protein systems have been shown to fuse synthetic and biomembranes.[7] Though fusion may also be induced using artificial membrane interactions,[8] there have been no reports of designed, controllable membrane fusion using well-defined small molecule recognition partners in apposing membranes. We report herein the induction of selective vesicle fusion with biological recognition motifs not natively associated with lipid bilayer fusion, thus broadening the scope of recognition-guided membrane activation.

Our system employs vancomycin glycopeptide and D-Ala-D-Ala-OH dipeptide as surface-bound fusogens. Vancomycin binds to peptides that C-terminate in D-Ala-D-Ala with micromolar dissociation constants through the formation of a hydrogen-bonded complex with D-Ala-D-Ala.[9] Membrane display of dipeptide and vancomycin derivatives[10] (**1** and **2**) was accomplished by coupling[11] to either phospholipid (**3**) or peptide (**4**) membrane anchors, respectively (Figure 1). Magainin 2, an antimicrobial peptide from frog skin, was chosen as an anchor for its ability to insert selectively into the hydrophobic matrix of negatively charged membranes and perturb lipid packing without vesicle fusion in the micromolar peptide concentration regime;[12] there are possibly many other natural and synthetic peptides that could serve this role.[13] We synthesized magainin itself as well as the vancomycin conjugate **4** (Figure 1) and confirmed that both the synthetic peptide and conjugates bind preferentially to negatively charged membranes[14] without significant change in vesicle size, as judged by dynamic light scattering (DLS). Indeed, initial screening by light scattering indicated that none of the individual compounds **1**−**4** caused appreciable change in size when incorporated at 1−5 mole percent, and therefore are not independently fusogenic.[4,5]

However, mixing dilute liposome preparations displaying complementary binding partners **3** and **4** in trans (different membranes) resulted in a rapid increase in scattering (size), followed by a slower size *decrease* over 1−2 h to reach a stable size population on average larger than the initial mean diameter. Typically, **3** and **4** were incorporated into large unilamellar vesicles (LUVs) at 1% surface concentration: **3** was incorporated in neutral phosphatidyl choline (egg PC) lipids (**3**-LUVs); **4** was surface-bound with 10% phosphoglycerol in egg PC (POPG, negatively charged) lipids (**4**-LUVs). Notably, preincubation of **3**-LUVs with free vancomycin suppressed changes in light scattering upon mixing with **4**-LUVs, strongly supporting the notion that liposome aggregation is mediated by molecular recognition between vancomycin and D-Ala-D-Ala.

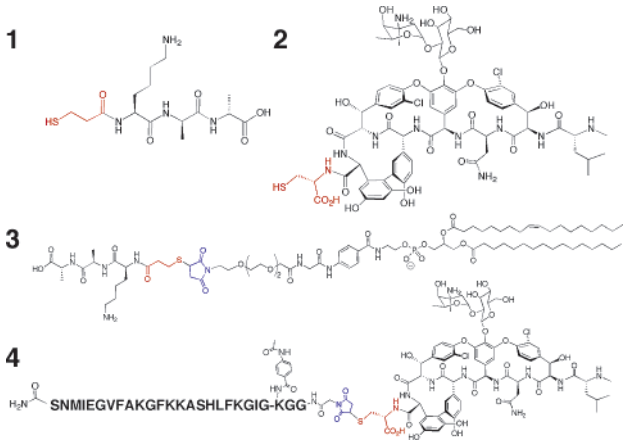


***Figure 1.*** Mercaptopropionamide−Kaa **1** and vancomycin−cysteine carboxamide **2** are reacted with maleimide-functionalized lipid and magainin anchors (red−blue connection) to yield **3** and **4**, respectively. The acetamidobenzamide moiety in **3** and **4** is a spectroscopic label.

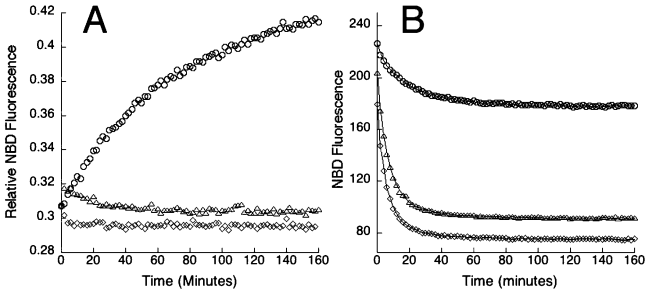


***Figure 2.*** Representative traces of NBD fluorescence. (A) NBD-PE and Rh-HDPE dilution assay of fusion. Vesicles with 2/1.5/1.5 mol % 3/NBD/Rh in egg PC were reacted with liposomes composed of 10 mol % POPG in egg PC that were (○) pretreated with 1 mol % **4**; (◇) pretreated with 1 mol % magainin (without vancomycin coupled); and (△) 1 mol % **4** in the presence of 5 equiv free vancomycin to **3**. (B) Inner monolayer mixing assay. NBD chemically quenched in outer monolayer of 2% **3**-LUVs mixed with (◇) 9 equiv, (△) 4 equiv, and (○) 1 equiv of LUVs bearing 1 mol % **4**.

A membrane fluorophore dilution assay for fusion was used in which a lipid-bound FRET pair, *N*-(7-nitroben-2-oxa-1,3-diazol-4-yl)amine (NBD-PE) and rhodamine (Rh-DHPE), was incorporated at 1.5 mol % concentration each in cis (same membrane) with **3**.[15] Fusion of these vesicles with unlabeled LUVs displaying **4** should cause fluorophore dilution in the larger volume of the product membrane, consequently increasing donor (NBD) and decreasing acceptor (Rh) fluorescence, respectively. Indeed, vesicle mixing resulted in this FRET fusion signature (Figure 2A); fusion began immediately upon mixing and slowed within 2 h to a stable population, mirroring DLS results. As with DLS, **1**−**4** and magainin itself were not fusogenic, and addition of free vancomycin blocked FRET change (Figure 2A). Furthermore, dose dependent fusion was observed with the addition of an excess of blank liposomes bearing



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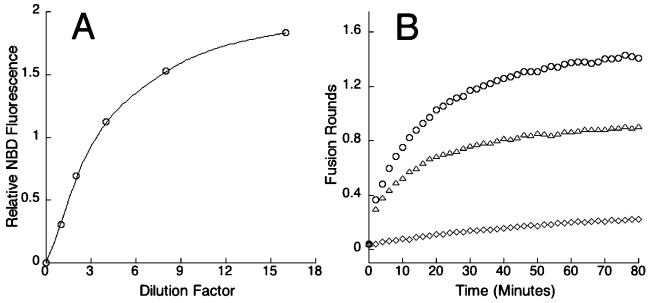


***Figure 3.*** (A) Rounds of fusion calibration curve. LUVs with 1:1 NBD/Rh prepared with increasing dilution to mimic FRET change caused by fusion. One round of fusion = dilution by a factor of 2. Less than one round of fusion represents a mixture of the liposomes at the original concentration and the one round dye diluted liposomes. (B) Rounds of fusion in dilution assay with (○) 9 equiv, (△) 4 equiv, and (◇) 1 equiv donor (blank) to acceptor (NBD/Rh) LUVs.

**4** to NBD/Rh/**3** labeled liposomes. More than one full round of fusion was observed with a 1:9 excess within 20 min, comparable to fusion rates displayed by native fusion systems in LUVs (Figure 3).[7a]

To distinguish this process from simple lipid mixing of the outer monolayers of each membrane, Rh/**4** LUVs were reacted with NBD/**3** LUVs in which NBD fluorophores on the outer monolayer were selectively destroyed by reduction with sodium dithionite,[16] which cannot effectively cross the membrane. FRET observed upon reaction of complementary recognition vesicles must result from the mixing of the *inner* monolayers that contain intact NBD, which is by definition membrane fusion. Gratifyingly, these experiments revealed a strong NBD/Rh FRET upon mixing of **3** and **4** derivatized liposomes, establishing this system as truly fusogenic (Figure 2B).

Fusion requires a surface charge differential between donor and acceptor LUVs; as 2% **3** in egg PC LUVs contributes only 1% net negative surface charge, magainin and **4** remain bound to the LUVs with 10% POPG, in trans to **3**. Liposome preparations in which *both* **3** and **4** containing LUVs had 10% PG lipids were non—or very weakly—fusogenic and nonaggregating as judged by light scattering and fluorescence measurements. One plausible explanation for this is that flattening of the charge gradient between the two liposome populations allows the magainin−vancomycin conjugate to equilibrate between the two membranes, leading to a decrease of membrane apposition and fusion rate.

These results indicate a fusion process in which surface binding initiates a highly aggregated state where fusion occurs rapidly and slows as the lipid binding partners increasingly occupy the same membrane: as membrane binding interactions in cis compete with interactions in trans, the number of binding partners available to catalyze membrane fusion decreases, as does the reaction rate and vesicular aggregation state. Interestingly, fusion rate is likely determined by the membrane location of the binding partner **4** and therefore the trans gradient of negatively charged lipids, which erodes as fusion proceeds. Further examination is underway to quantify the behavior of this system and evaluate its potential as a selective delivery vehicle. This initial study establishes the controllable and biomimetic ability of designed fusogens to activate specific membrane mergers in synthetic membranes via small-molecule recognition; this has resonance with goals in targeted chemical delivery and nanoscale compartmentalized chemistry.

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**Supporting Information Available:** Synthetic procedures, light scattering and fluorescence data. This material is available free of charge via the Internet at http://pubs.acs.org.

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