Formation of Supported Lipid Bilayer Composition Arrays by Controlled Mixing and Surface Capture

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Virtually all structures in living cells are defined by lipid bilayers. Many molecular components can diffuse along these 2-dimensional structures, sampling all or part of the membrane, and this diffusion is central to their function. Supported lipid bilayers capture this membrane fluidity and have been used to study a wide range of membrane-associated biomolecules.[1−4] Recently, several techniques have been developed to microfabricate barriers on glass substrates to direct lipid bilayer self-assembly, facilitating the patterning of multiple, isolated membrane regions, or corrals, on a single substrate.[5−12] The resultant corrals are typically of identical composition; however, a handful of strategies, including spatially directed photochemical transformations[11] and membrane stamping,[8,13] can be used to produce bilayer arrays with specific composition variations in different corrals. These composition arrays are desirable for investigating numerous biological systems, including how lipid and/or protein composition affects binding of membrane-associated receptors with their ligands or each other, cellular processes such as intracellular protein modification and trafficking, and cell activation. In this communication, we introduce a robust approach that, for a wide variety of situations, greatly simplifies the production of large-scale parallel bilayer arrays with defined variations in composition.

The method, outlined in Figure 1, is based on exposing a pre-patterned surface to a gradient of vesicles of various compositions created by flow and taking advantage of irreversible binding of vesicles to a substrate, subsequent fusion into a bilayer, and lipid mixing within each corralled region. For demonstration purposes, we fabricated a simple, millimeter-scale, converging channel out of poly(dimethylsiloxane) (PDMS, Sylgard 184; Dow Corning) elastomer (Figure 1A). One wall of the channel consisted of a glass coverslip containing microcontact-printed[9,10,14−16] grid lines of fibronectin;[17] however, any of a variety of materials can be used to create these barriers.[5,8,10,12] The elastomer channel was filled with water, and then two solutions of vesicles were manually pumped through the channel as illustrated in Figure 1B. For visualization purposes, each solution contained vesicles supplemented with a small amount of a different fluorescently labeled lipid;[18] conceptually, this technique is compatible with any biomolecule that can be incorporated into lipid bilayers by vesicle fusion. Downstream of the convergence, the two solutions partially mix by diffusion,[19] exposing the micropatterned array to a controllable, spatially defined gradient of the two types of vesicles.

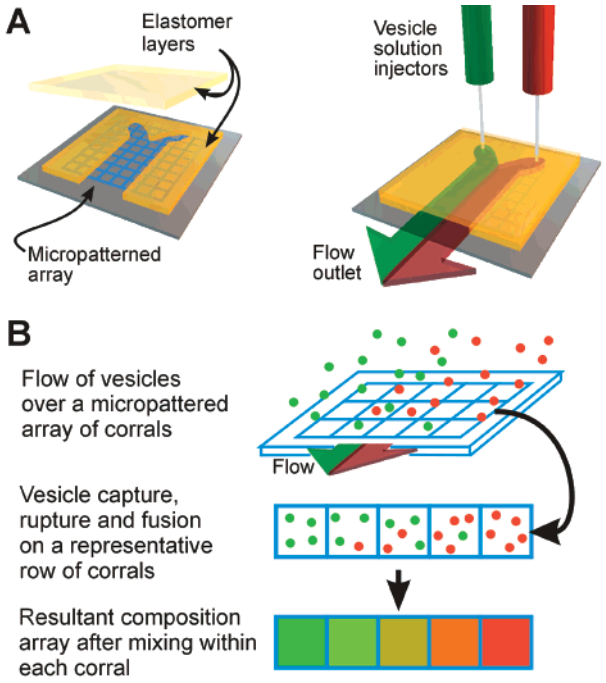

Figure 1. (A) Schematic illustration of the converging flow configuration used to produce limited mixing of two types of vesicles in solution. Two layers of poly(dimethylsiloxane) elastomer were cut using a template, oxidized in an air plasma, and layered to produce three walls of the converging channel (the channel downstream of the convergence measured $1 \times 5 \times 10$ mm^3). A glass coverslip patterned with an array of corrals[17] comprised the fourth wall of the channel. (B) Schematic diagram illustrating controlled mixing and surface capture onto a substrate subjected to the flow shown in panel A. The local composition of a supported lipid bilayer formed by vesicle fusion reflects the makeup and spatial distribution of vesicles (denoted here as red and green dots) that are flowed past a pre-patterned surface. This compositional profile is spatially averaged as the components within each corral diffuse and mix.

The vesicles are irreversibly captured on the micropatterned coverslip, subsequently rupturing and fusing into a continuous supported lipid bilayer.[3,4,21] The local composition of the bilayer reflects the distribution of vesicles exposed to the surface. Because the bilayer is a 2-dimensional fluid, any nonuniformity in membrane composition will be erased by lateral diffusion.[10,11] The presence of barriers, however, limits this mixing to defined

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(17) The use of proteins as barrier materials can confer additional biological activity to patterned lipid bilayer surfaces; we have used fibronectin barriers to promote adhesion of anchorage-dependent cells onto lipid bilayer substrates, directing the interaction of these cells with the membrane patches.[9] Here, fibronectin was labeled with Cascade Blue (Molecular Probes) to facilitate visualization of the micropatterned barriers.

(18) Small unilamellar vesicles (SUVs) were prepared from dilauroylphosphatidylcholine (DLPC.; Avanti) supplemented with either 1 mol % of Texas-Red DHPE (TR-DHPE.; Molecular Probes), 2 mol % of NBD-DHPE (Avanti), or 0.5 mol % of DiD (Molecular Probes). Lipids were reconstituted in water (5 mg lipids/mL) then extruded[10] through 50-nm-pore membranes (Avanti). These stock solutions were diluted 1:3 (v/v) in aqueous buffer (pH 7.0) prior to use. Images of fluorescently labeled lipids and proteins were collected using appropriate filter sets, and are presented in false color as indicated in the figure legends.

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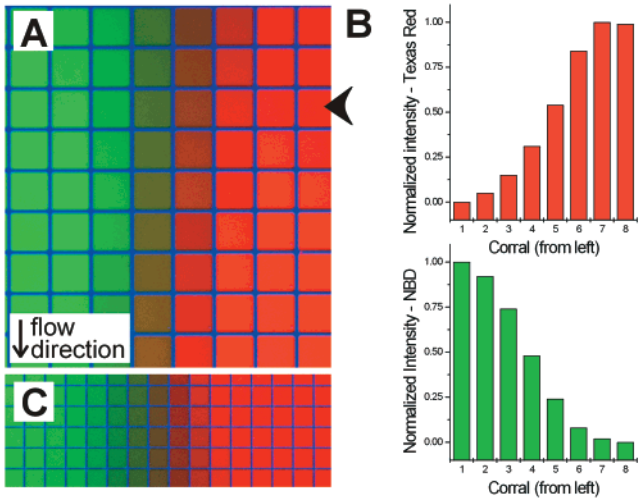

Figure 2. Epifluorescence micrograph illustrating composition arrays prepared using the approach outlined in Figure 1. (A) An array of 50 × 50 μm^2 corrals made from Cascade Blue-labeled fibronectin[17] (shown in blue). Solutions of vesicles[18] supplemented with either NBD-DHPE (green) or TR-DHPE (red) were flowed over this surface at a combined rate of ~1 μL/s. The vesicle solution was flowed over this surface at a small angle to the columns of corrals, resulting in composition gradients both along and across the direction of flow. (B) The average, normalized fluorescence measured in each of the corrals in the row indicated by the black arrow in panel A. (C) Reducing the size of the lipid corrals to 25 × 25 μm^2 produces a finer gradation in lipid composition.

regions of the surface. Consequently, the composition of each corral reflects the local *average* composition of the vesicle solution, as illustrated schematically in Figure 1B. The spatial distribution or gradient of vesicles in a solution flowed over a micropatterned surface is thus captured or binned by the surface.

A typical surface is shown in Figure 2A. The composition gradient is readily observed by eye and is quantified by fluorescence microscopy (Figure 2B), demonstrating that even this simple converging flow configuration and injection system produces a well-defined concentration gradient. Membrane fluidity is evidenced both by the attainment of uniform fluorescence intensity within each lipid corral, with systematic variations in corral-to-corral composition, and by a direct demonstration of lateral mobility. Figure 3 shows a concentration array containing varying amounts of two oppositely charged, dye-labeled lipids.[18] Upon application of an electric field parallel to the membrane surface, these lipids (which comprise only a small fraction of the lipids in the membranes) move toward opposite sides of each corral and can be visualized separately.[7] Upon removal of the field, the lipids diffuse freely and remix within each corral (not shown).

The interaction between the vesicle solution flow and the micropatterned surface can be modulated to change the resolution and/or extent of these concentration arrays. For example, if the flow impinges on the micropatterned surface at a small angle to a column of corrals, as is the case in Figure 2A, a large number of intermediate concentrations are obtained in the direction of flow; this off-angle flow increases both the extent and resolution of the composition array. Decreasing the size of each corral is an alternative approach to increasing the composition resolution, but without increasing the extent of the array. This is illustrated in Figure 2C, which shows an array of 25 × 25 μm^2 corrals subject to the same flow parameters as in Figure 2B. It is also straightforward to modulate mixing between the two vesicle solutions by, for example, changing the flow rate, flow pattern, and/or relative concentrations of vesicles in each solution, leading to considerable control over the composition perpendicular and parallel to the direction of flow. Adaptation of microfluidics approaches[22] to tailor complex, well-defined concentration profiles of multiple components (using multiple solution injectors) could be used to produce increasingly sophisticated membrane arrays.

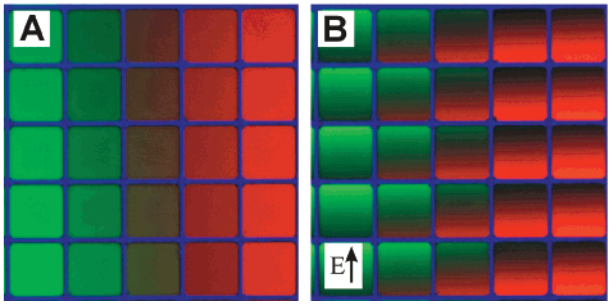

Figure 3. Epifluorescence micrograph of a concentration gradient of DLPC supplemented with TR-DHPE (negatively charged and shown in red) and DiD (positively charged and presented in green) (A) before and (B) 10 min after application of an electric field of 40 V/cm in the direction indicated in panel B. The two charged lipids migrated toward opposite sides of, but remained within, each corral. These corrals each measure 50 × 50 μm^2.

The salient features of the technique presented here are that composition arrays containing hundreds of membrane corrals are readily produced in a single step and that a wide range of compositions can be made using only two vesicle preparations without generating a separate solution for each intermediate composition. Both of these factors greatly simplify the preparation of membrane composition arrays. Moreover, each membrane corral is not individually addressed during production of the array, but can be individually probed using conventional fluorescence microscopy or standard fluorescence chip readers. Miniaturization of the corral dimensions (with a concurrent increase in array density and composition resolution) is thus readily feasible and limited only by the detection strategy. Lastly, the method described here is generally applicable for use with any compound that can be incorporated into lipid bilayers by vesicle fusion, providing a robust technique for preparing composition arrays of a wide variety of membrane-associated biomolecules.

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(19) Under the conditions used in this communication, flow within the millimeter-scale, converging channel is essentially laminar (Reynolds Number ~70). Mixing between the two vesicle solutions is thus limited by diffusion. The no-slip boundary condition at each wall dictates that the flow velocity at the interface between the two solutions immediately after the junction is lower, with thus a longer residence time, compared to that downstream.[20] Consequently, mixing as a function of distance is greater immediately after the junction compared to that downstream of the confluence.

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