MembraneChip™ : Arrays of Natively Displayed Membrane Targets for Novel Drug Discovery

V Yamazaki, LT Nguyen, RJ Schafer, N Ulman, JT Groves*

Proteomic Systems, Inc.
Sunnyvale, CA 94086

ABSTRACT

Naturally fluid and functional biological membranes have been integrated into a scaleable system. Previously-inaccessible membrane targets can be presented in their native environment. Domains of membrane targets naturally displayed in cell membranes can be exposed for drug discovery. The MembraneChip™ is an array of 16-64 distinct membrane targets in their fluid lipid bilayer environment. Each lipid bilayer expanse is corralled on a micron scale. MembraneChips™ have been made compatible with standard well plate systems. Its architecture achieves natural cell membrane properties in a scalable and controllable configuration for novel drug discovery.

Keywords: membrane, array, discovery, lipid, bilayer.

INTRODUCTION

The astonishing increase in genomic information has jump started the understanding of the proteome. Splice signals delineating the proteins are just starting to be understood in addition to signals for protein modifications determining structure and function. Studies of soluble proteins in microarray formats are becoming the norm, yet concurrently a paucity of technology focusing on membrane targets exits [1]. Membranes organize the functional components of each human cell, and furthermore, represent one of the most important entities especially for drug developers as a large percentage of targets for current therapeutics reside in this structure. Many initial signaling events take place here as ligands bind to plasma membrane receptors. The activation of tyrosine kinases, serine/threonine kinases, and G-protein coupled receptors eventually result in a variety of cellular phenotypes [2].

Cell membrane is visualized as a dynamic fluid mosaic with continuously moving and reorganizing residents of the lipid bilayer. The supported membrane or supported bilayer system captures the natural characteristics of a real membrane. It consists of a fluid and continuous single lipid bilayer on a solid substrate [3, 4] (Figure 1). By a variety of basic forces the lipid bilayer floats above the substrate with 1nm of water layer separating the two [4]. The mobile membrane components demonstrate long range lateral mobility as a direct consequence of this lack of physical contact. This key life-like feature differentiates this system from all others. It allows observation of the dynamic interactions between cells.

Vital to higher organisms, communication between cells takes place in synapses. Directed secretion across these stable adhesive junctions transduces information from one cell to another [5]. These specialized junctions play an important role when antigen-presenting cells activate T lymphocyte cells. The determinative pattern of a central cluster of T cell receptors surrounded by a ring of adhesion molecules was determined by replacing the antigen-displaying cells with a supported bilayer displaying MHC and ICAM [6]. A supported membrane system has clearly aided in elucidating the specific interactions involved in an immune synapse.

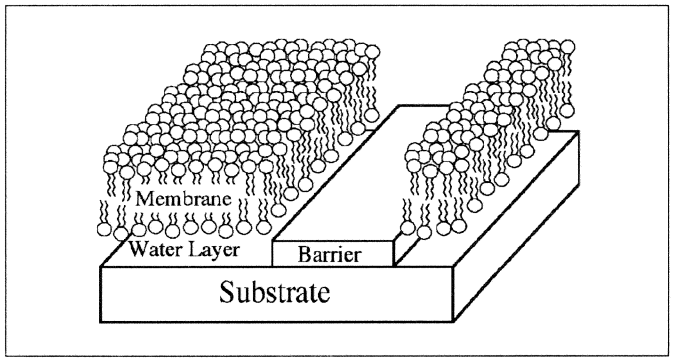

Figure 1 Schematic of a Supported Membrane
A thin layer of water separates the continuous fluid bilayer from the substrate. Barriers patterned onto the substrate corral the bilayers to form discrete membrane arrays. (Reprinted with the permission from American Association for the Advancement of Science and Groves JT Ulman N, Boxer SG, *Micropatterning fluid lipid bilayers on solid supports.* Science, 1997. 275: p. 651-653. © 1997 American Association for the Advancement of Science.)

* To whom correspondence should be addressed. Email: jtgroves@proteomicsys.com

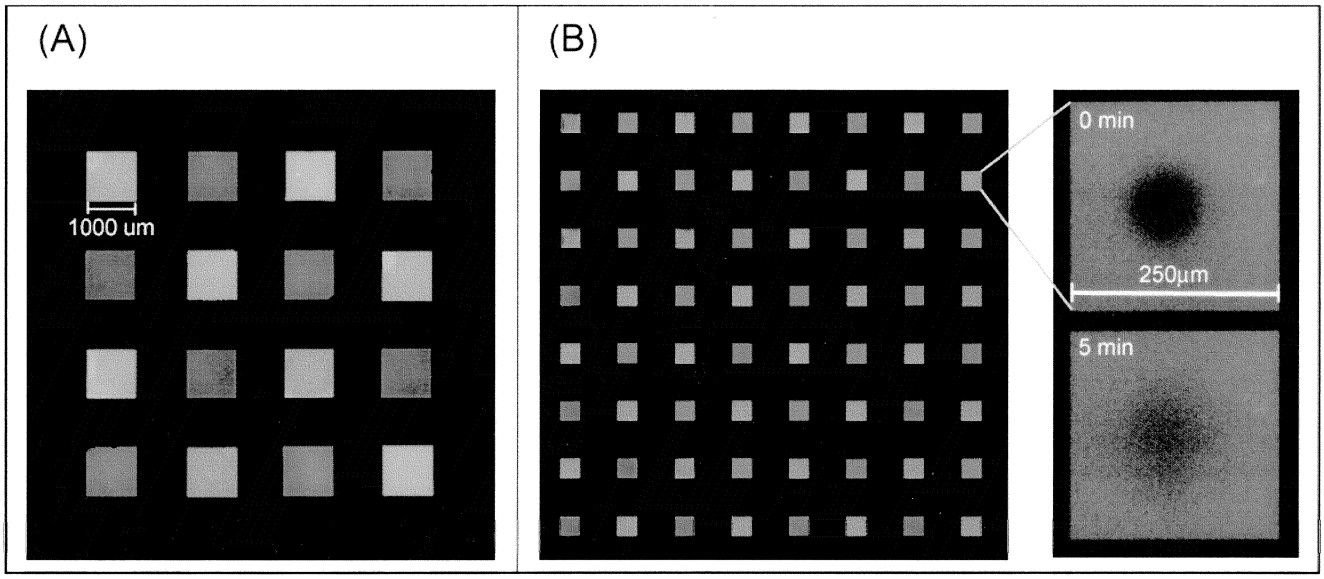

Figure 2. MembraneChips™: Fluid Membrane Arrays
(**A**) Patterned chrome barriers on silicon oxide corral membrane compatible regions of 1000µm^2. Corrals are addressed with two different vesicles: egg phosphatidylcholine with 1 percent mole N-(Texas Red sulfonyl)-1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine (red) and egg phosphatidylcholine with 1 percent mole 16:0-12:0 NBD-phosphatidylglycerol (green). (**B**) Advanced microarraying technology takes approximately 60 seconds to array out a few nanoliters of red and green lipid vesicle solutions into 64 250µm^2 corrals. The 8x8 array elements displays more homogeneity with absolutely discrete elements compared to some mixing occurring with the hand arrayed chip (Figure 2A). Fluorescence recovery after photobleaching (FRAP) is a standard method to test lateral mobility of lipids. One representative corral from a 8x8 chip shows fluidity (Figure 2B). Excitation light shadows a small area of the corral for 1 min, photobleaching the fluorescent molecules in that area (Figure 2B, right top). 5 min after closing off the light source, fluorescent molecules surrounding the area continuously diffuse back into the photobleached area, diminishing the original shadow (Figure 2B, right below).

MEMBRANE ARRAY TECHNOLOGY AND APPLICATIONS

Frequently supported bilayers are constructed from fusion of unilamellar lipid vesicles with membrane compatible surfaces, such as silicon oxide [3, 4]. A combination of physical forces, including van der Waals, electrostatic, and hydration, stabilize the single lipid bilayer in a plane with 1nm of water above the solid support [3] (Figure 1). This basic supported membrane system has found a great number of applications in the academic setting. [3, 7-10] This mature research technology has just begun its transformation into an industrial drug discovery platform, bringing with it two levels of advantages. One is the sheer force of multiplexing, common to all array technologies [1, 11-13]. Furthermore, even on the level of an individual element the membrane array technology differs intrinsically from all others [14]. This system captures the essence of biological and functional characteristics of membranes by retaining their fluidity. So far it is the most life-like system amenable to drug discovery programs.

This amenability comes from being a true hybrid technology of materials science and cell biology. The central issue in forming arrays is exactly how to control the fluid bilayer elements. As the word implies, a fluid membrane mixes and moves. One solution involves the discovery of lipid bilayer incompatible materials for barriers [4]. A wide variety of metals (Au, Al, Cr, Ti, etc), metal oxides (Al_2O_3, TiO_2), and some polymers can serve effectively as barriers (reviewed in [14]). These barrier grids can confine fluid membranes into discrete array elements (Figure 2). With a dexterous scientist the limit of corrals addressable manually is 1000µm^2 (Figure 2A). Any smaller array sizes can be addressed only with advanced microarraying technology (250µm^2, Figure 2B). Nanoliters of lipid vesicle solutions can be rapidly ejected into each membrane compatible corral [15, 16]. Not only is there an increase in homogeneity in corrals but the membrane elements remain absolutely discrete as demonstrated by arraying green and red vesicles in alternating corrals (Figure 2B). Furthermore, the machine arrayed membranes retain lateral mobility as tested by fluorescence recovery after photobleaching (FRAP), which is the most commonly utilized method to monitor membrane fluidity [9, 17]. FRAP confirms fluidity on one representative 8x8 membrane element (Figure 2B). Excitation light shadows a small area of the corral, photobleaching the fluorescent

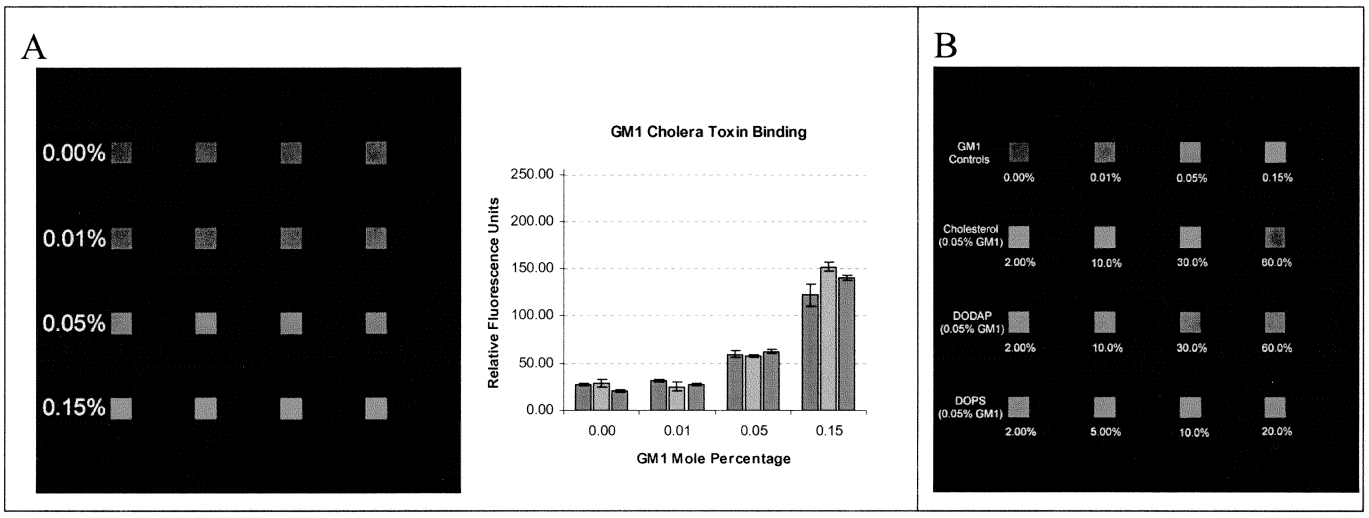

Figure 3. Membrane Array Technology and Applications
MembraneChip™ holds the potential for various applications. (**A**) Membrane compositions containing different concentrations (percent mole) of ganglioside G_{M1} in egg phosphatidylcholine with 1 percent mole 16:0-12:0 NBD-phosphatidylglycerol are displayed in quadruplicate. Three identical chips are constructed, and the raw relative fluorescence units are displayed after exposure to Cholera Toxin B subunit labeled with Alexa Fluor 594, demonstrating good inter and intra chip reproducibility. (**B**) MembraneChips™ can be used to study effects of different lipid compositions on cholera toxin subunit B and ganglioside G_{M1} binding.

molecules in that area. After closing off the light source, fluorescent molecules surrounding the area continuously diffuse back into the photobleached area, diminishing the original shadow.

Various discovery opportunities can be imagined with discrete fluid lipid membrane arrays. To demonstrate this potential, proof of concept experiments are performed with cholera toxin and ganglioside G_{M1}. The recruitment of a lipid bilayer resident, ganglioside G_{M1}, by pentameric cholera toxin is facilitated by the intrinsic fluidity displayed in our system. Pentameric cholera toxin binds one ganglioside G_{M1} in each of its subunits [18, 19]. Three 4x4 chips with 500μm^2 corrals display different concentrations of G_{M1} in quadruplicate (Figure 3A). Not only are the fluorescence measurements in quadruplicated corrals in close agreement with each other, but agreement is also seen among chips (Figure 3A). Furthermore, membrane targets can easily be reconstituted in different lipid environments (Figure 3B). CXCR4 receptors in T cell lines cannot bind ligand, mediate calcium fluxes or undergo internalization when cholesterol is depleted from the cell membrane [20]. Nguyen and Taub demonstrate the significance of lipid composition for G-protein coupled receptor function.

CONCLUSION

The membrane array technology captures the natural properties of membranes by preserving their characteristic lateral fluidity, making it intrinsically different from any other technology. Great advancements have been made in corralling these fluid bilayers to form discrete arrays. A drug discovery platform is emerging with automated arraying techniques. Now screening is possible with a system incorporating basic and natural characteristics of the membrane.

REFERENCES

1. Mitchell P, *A perspective on protein microarrays.* Nat Biotechnol, 2002. **20**: p. 225-229.
2. Leof EB, *Growth factor receptor signalling: location, location, location.* Trends Cell Biol, 2000. **10**: p. 343-348.
3. Sackmann E, *Supported membranes: scientific and practical applications.* Science, 1996. **271**: p. 43-48.
4. Groves JT, Ulman N, Boxer SG, *Micropatterning fluid lipid bilayers on solid supports.* Science, 1997. **275**: p. 651-653.
5. Dustin ML, Colman DR, *Neural and immunological synaptic relations.* Science, 2002. **298**: p. 785-789.
6. Grakoui A, Bromley SK, Sumen C, Davis MM, Shaw AS, Allen PM, Dustin ML, *The immunological synapse: A molecular machine controlling T-cell activation.* Science, 1999. **285**: p. 221-227.
7. Bayley H, Cremer PS, *Stochastic sensors inspired by biology.* Nature, 2001. **413**: p. 226-230.
8. Dietrich C, Bagatolli LA, Volovyk ZN, Thompson NL, Levi M, Jacobson K, Gratton E, *Lipid rafts reconstituted in model membranes.* Biophys J, 2001. **80**: p. 1417-14.

9. Groves JT, Boxer SG, *Micropattern formation in supported lipid membranes.* Acc Chem Res, 2002. **35**: p. 149-157.
10. Sackmann E, Tanaka M, *Supported membranes on soft polymer cushions: fabrication, characterization and applications.* Trends Biotechnol, 2000. **18**: p. 58-64.
11. Wagner P, Raymond K, *Protein biochips: An emerging tool for proteomics research.* Curr Drug Disc, May 2002: p. 23-28.
12. MacBeath G, Schreiber SL, *Printing proteins as microarrays for high-throughput function determination.* Science, 2000. **289**: p. 1760-1763.
13. Fodor SP, Read JL, Pirrung MC, Stryer L, Lu AT, Solas D, *Light-directed, spatially addressable parallel chemical synthesis.* Science, 1991. **251**: p. 767-773.
14. Groves JT, *Membrane array technology for drug discovery.* Curr Drug Disc, 2002. **5**: p. 606-612.
15. Boxer SG, Ulman N, Groves JT, *Arrays of independently-addressable supported fluid bilayer membranes*. 2001: US Patent 6,228,326.
16. Yamazaki V, Ulman N, Nguyen LT, Boxer SG, Groves JT, *Biosensor arrays and methods*. 2002: US Serial Number 10/200,682.
17. Tamm LK, Kalb E, *Microspectrofluoremetry of supported planar membranes*, in *Molecular Luminescence Spectroscopy*, S. SG, Editor. 1993, John Wiley & Sons Inc: New York, NY. p. 253-305.
18. Schon A, Freire E, *Thermodynamics of intersubunit interactions in cholera toxin upon binding to the oligosaccharide portion of its cell surface receptor, ganglioside GM1.* Biochemistry, 1989. **13**: p. 5019-5024.
19. Merritt EA, Sarfaty S, Van Den Akker F, L'Hoir C, Martial JA, Hol WGJ, *Crystal structure of cholera toxin B-pentamer bound to receptor GM1 pentasaccharide.* Protein Sci, 1994. **3**: p. 166-175.
20. Nguyen DH, Taub D, *CXCR4 function requires membrane cholesterol: implications for HIV infection.* J Immunol, 2002. **168**: p. 4121-4126.