Anisotropic Diffusion in Nanopatterned Supported Lipid Bilayers

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Statement of Purpose: Membrane-associated, cell-cell communication proteins often exhibit long-range diffusion along the cell surface that is much slower than predicted based on the properties of the plasma membrane alone. This hindered diffusion possibly results from the interaction of proteins with biomolecular barriers that either comprise or are associated with the cell cytoskeleton. Towards an understanding of how hindered diffusion modulates cell-cell communication, we created nanopatterned, substrate-supported lipid bilayers containing barriers that mimic the spacing of cytoskeletal structures (Fig. 1). Specifically, these patterns consist of long, parallel barriers interspersed with gaps that connect adjacent regions of bilayer; lipids diffuse freely parallel to the barriers, but encounter barriers that hinder, but do not completely block, diffusion in the perpendicular direction. In this report, we compare long-range diffusion of lipid bilayers formed on these nanopatterned surfaces as function of barrier geometry.

Methods: Nanopatterned surfaces contained arrays of titanium barriers defined as in the figure at the top of the next column. In this report, two line spacings (ls) were considered – 125 and 250 nm. Three gap sizes were also examined – 30, 40, and 50 nm. In all cases, the barriers were 50 nm wide, and the gaps were spaced 500 nm center-to-center. Control surfaces consisted on non-patterned glass as well as glass patterned with continuous barriers (gap=0), spaced 250 nm apart.

Glass coverslips were nanopatterned using a standard lift-off procedure. Coverslips were cleaned by immersion in detergent, baked at 450 C for 6 hours, then soaked in Nanostrip cleaner for 40 minutes. Substrates were spin-coated with a layer of 25kDa PMMA A3 followed by 950 kDa PMMA A2.5, for a total thickness of ~ 200 nm. A discharge layer of AquaSave (Mitsubishi) was then applied, and the desired pattern of dashed lines written using an electron beam system. Following development, a 4-nm layer of Titanium metal was deposited by ebeam evaporation over the entire substrate. Finally, resist and overlying metal were removed in acetone.

Lipid bilayers were formed by vesicle fusion. Briefly, small unilamellar vesicles of egg phosphatidylcholine (Avanti Polar Lipids), supplemented with 1% (mol/mol) of Texas-Red DHPE (Invitrogen), were prepared by extrusion through membranes containing 50-nm pores. Vesicles were exposed to prepared substrates for 10 minutes to allow formation of the lipid bilayer. Substrates were washed extensively with PBS and water.

Anisotropic, long range diffusion coefficients were calculated using fluorescence recovery after photobleaching (FRAP). A roughly 50 μm-wide photo bleach spot was formed in each supported bilayers by aperatured exposure to illumination light. Fourier

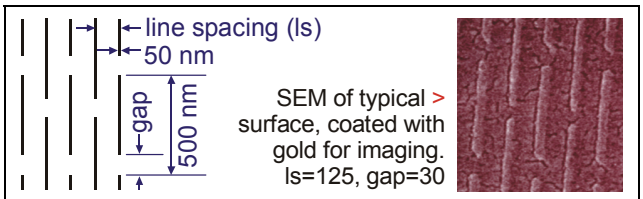

Figure 1: Nanofabricated barriers to lipid diffusion.

analysis of a timeseries of images recording the recovery of this spot was used to determine long-range diffusion coefficients of the Texas-Red-labeled lipids parallel to and perpendicular across the barriers. Data were analyzed using ANOVA methods; multiple comparisons were done using Tukey critera (α=0.05).

Simulation of diffusion was carried out in the MATLAB software package, using the PDE toolbox.

Results/Discussion:

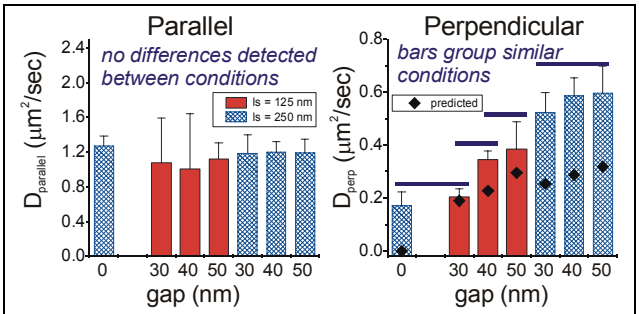

Figure 2: Measured diffusion coefficients

- Long-range diffusion parallel to the barriers, as measured by FRAP analysis of Texas-Red labeled lipids, was similar across all patterned surfaces, and identical to that on unpatterned glass (D=1.1 μm^2/sec). This result suggests that short-range diffusion was identical across surfaces and unaffected by the barriers.
- Long-range diffusion perpendicular to the barriers was significantly lower (as measured by diffusion coefficient) with decreasing line spacing (ls) and gap size.
- However, measured diffusion coefficients differed from those predicted using continuum models. Along with the observation of diffusion across the continuous barriers (gap = 0), this suggests a more complex interaction of the bilayer with the barriers. This topic is currently under investigation.

Conclusions: We introduce a new supported lipid bilayer model that captures the complex differences between short- and long-range diffusion of proteins observed on living cells. This model will be valuable in future studies of cell-cell communication and membrane proteins.

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