Material Properties of Lipid Micro-Domains: Force Volume Imaging Study of the Effect of Cholesterol on Lipid Micro-Domain Rigidity

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Materials and Methods

1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), 1,2-dioleoyl-sn-glycero-3-phospho-L-serine (DOPS), N-heptadecanoyl-D-erythro-sphingosylphosphorylcholine (C17 sphingomyelin, SM) and cholesterol (CHOL) were purchased from Avanti Polar Lipids (Birmingham, AL, USA). All lipids were >99% pure. For all experiments Milli-Q water was used. HEPES was purchased from Sigma.

Vesicle Preparation

All lipids were first dissolved in chloroform and mixed at different ratios. The ratio of DOPC, DOPS, and SM was 1:1:2 (mol/mol/mol) and cholesterol concentrations were 0, 10, 20, 25, 33, and 50% of the total amount of lipid. SLBs of SM and of DOPC and DOPS (1:1, mol/mol) with/without cholesterol were also used. The lipid mixture was dried under nitrogen gas, followed by vacuum evaporation for 3 h prior to being suspended in 10 mM HEPES buffer, 150 mM NaCl (pH 7.0) (1). The final concentration of lipids was 1 mM. Samples were left to hydrate overnight above phase transition temperature (T_m), followed by sonication for 30 min. During sonication, periodic vortex mixing was carried out prior to vesicle extrusion (Avanti Mini-Extruder, Avanti Polar Lipids, Birmingham, AL, USA) 20 times through a polycarbonate membrane filter of defined pore diameter, typically 100 nm. Extrusion was performed at temperatures above the transition temperature of the component sphingomyelin, since gel-state lipids are difficult to extrude at lower temperatures (2).

Supported Lipid Bilayer (SLB) Preparation

Formation of SLBs was achieved by the vesicle fusion method. 100µL of 100nm small vesicle solution (10mM HEPES, 150mM NaCl, 20mM $CaCl_2$, pH 7.0) was deposited onto a freshly cleaved mica surface. The sample was incubated for 2h at room temperature. Then the sample was rinsed 10 times with 10mM HEPES, 150mM NaCl, pH 7.0 to remove excess vesicles and calcium salts. Afterwards, the sample was placed on a heating stage, incubated for 1h at 55 ^{o}C (3). The sample was left to cool down to room temperature and rinsed 10 times with the same buffer mentioned previously prior to imaging.

AFM FV Imaging

The visualization and force measurement of supported bilayers were performed using a commercial AFM (Nanoscope IV, Digital Instruments/Veeco, Santa Barbara, CA) equipped with a J scanner. A quartz fluid cell was used without the o-ring. The samples were imaged by tapping mode using unmodified triangular Si_3N_4 cantilevers (OTR8, Digital Instruments) with a nominal spring constant of 0.15 N m^{-1} operating at the frequency of 7-9 kHz in tapping

mode first and changed to contact mode to perform FV imaging. The apparent height and size of observed domains were studied with the analysis function of the Nanoscope software (Version 5.30r3sr3, Digital Instruments/Veeco, Santa Barbara, CA). The spring constant of the cantilever was calibrated using a standard cantilever approach (4). The force constant of the standard was estimated by the method developed by Sader et al. (5). The measured spring constants were 10-20 % higher than the manufacturer's nominal value with 10% error. Force spectroscopy was analysed by AFM Force Volume data analysis software (Version 1.7, Textile and Fibretechnology, Belmont, Victoria). FV imaging was conducted under higher ionic strength buffer (10mM HEPES, 150mM NaCl, pH 7.0) and lower ionic strength buffer (10mM HEPES, 1mM NaCl, pH 7.0) at room temperature.

FV images (32 x 32 force curves) were collected at a scan rate of 0.0723Hz, in relative trigger mode (6). The trigger value is the turning set point when doing force curves and corresponds to the maximum load applied where the approach stops and retract begins. Different trigger values were set to measure the threshold force to puncture to bilayers. The AFM imaging system was stabilized and the sample was imaged by contact mode at the minimal load possible, usually less than 1 nN before FV experiments. All force curve measurements acquired 256 sample data points. Interactions between probe and bilayer during approach and withdrawal were analysed with Nanoscope software. Approximately 200 force curves inside domains were used for surface analysis.

Additional Figures and Tables

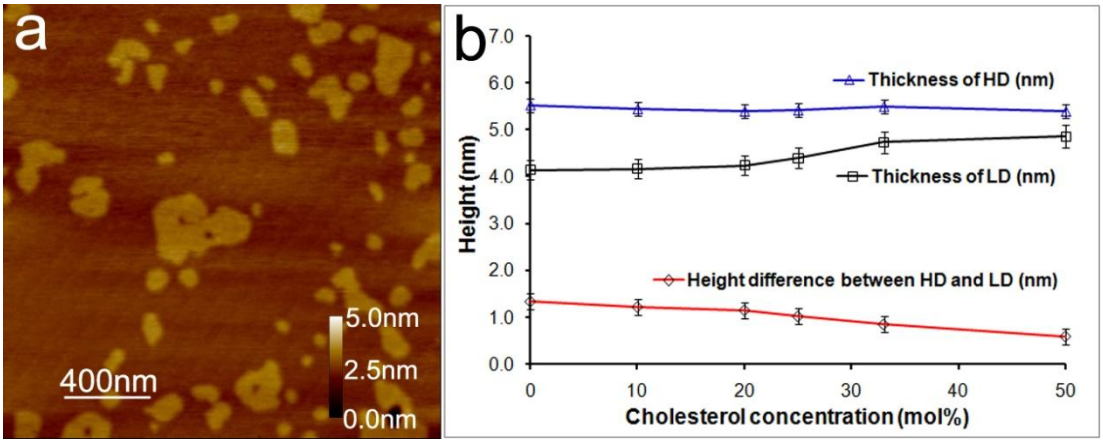

FIGURE S1 (a). AFM height image of DOPC/DOPS/SM (1:1:2, mol/mol/mol) without cholesterol under high ionic strength buffer. Scan size: 2μm×2μm, height scale: 5 nm. (b) The height difference between the higher domain (HD) and the lower domain (LD) as well as the thickness of HD and LD with increasing concentration of cholesterol (n>30).

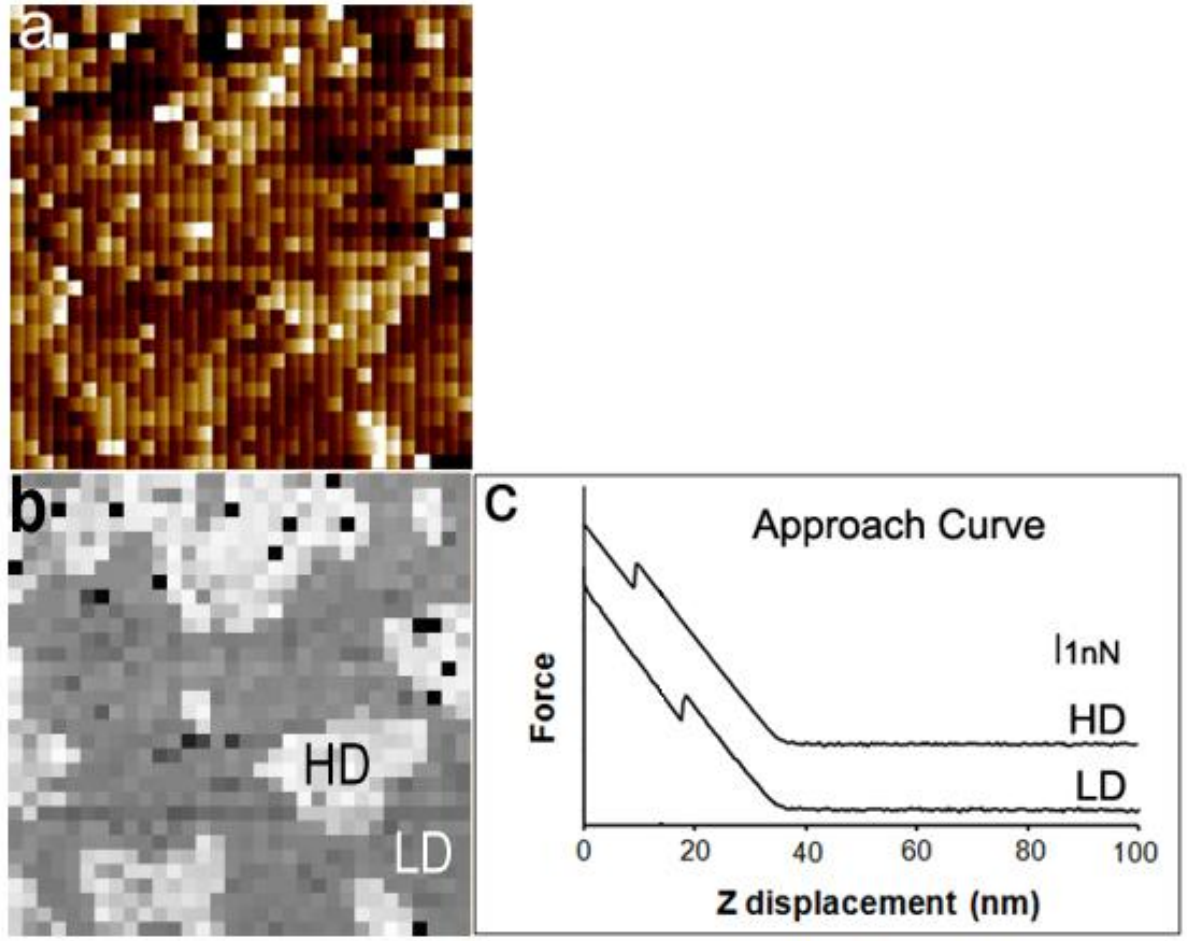

FIGURE S2 Force Volume imaging using trigger values (7.2 nN) above that required to breakthrough the SM-rich domains of the bilayer on DOPC/DOPS/SM (1:1:2, mol/mol/mol) bilayers containing 10 mol% cholesterol under high ionic strength solutions. (a) AFM height image. Scan size: 500nm×500nm, height scale: 5 nm. (b) Breakthrough map created using approach force curves such as those shown in (c), force scale: 7.2 nN. (c) Force spectra showing a higher breakthrough force corresponding to lighter pixels in (b) (HD) and a lower breakthrough force corresponding to darker pixels in (b) (LD).

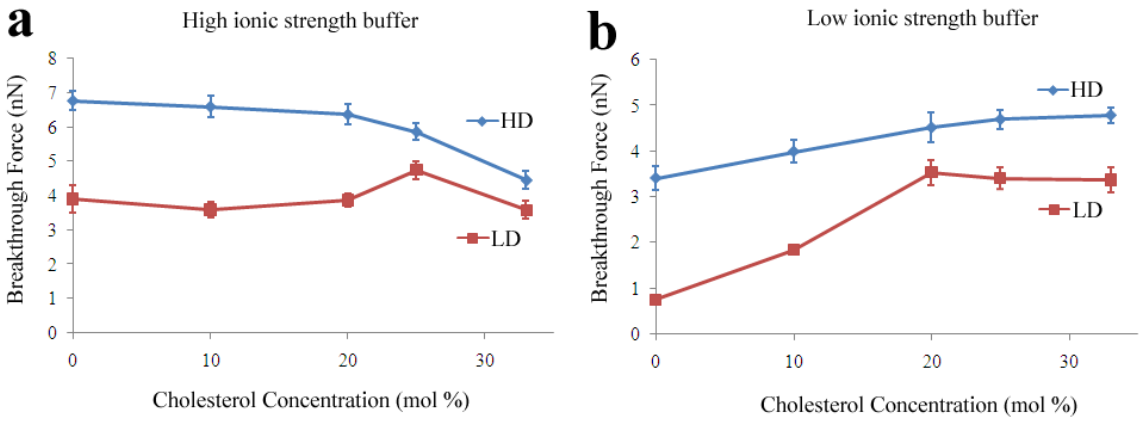

Figure S3 Breakthrough forces on DOPC/DOPS/SM (1:1:2, mol/mol/mol) bilayers containing different concentrations of cholesterol (0-33 mol%) measured under high (a) and low (b) ionic strength conditions. Errors presented correspond to one standard deviation (±).

	Cholesterol concentration (mol%)	High ionic strength buffer (nN)		Low ionic strength buffer (nN)	
		HD	LD	HD	LD
DOPC/DOPS (1:1, mol/mol)	0	4.38(0.51)		3.58(0.64)	
	10	5.07(0.27)	4.07 (0.21)	4.22(0.41)	3.17(0.44)
SM	0	6.60(0.50)		0.78(0.04)	
	25	6.21(0.84)	4.28(0.57)	-	-
DOPC/DOPS/ SM (1:1:2, mol/mol/mol)	0	6.77(0.58)	3.90(0.78)	3.41(0.54)	0.76(0.06)
	10	6.60(0.61)	3.60(0.46)	3.99(0.50)	1.84(0.20)
	20	6.37(0.61)	3.87(0.35)	4.51(0.65)	3.53(0.55)
	25	5.87(0.50)	4.74(0.53)	4.69(0.41)	3.41(0.47)
	33	4.46(0.53)	3.59(0.53)	4.78(0.32)	3.37(0.57)

Table S1 Gaussian fit parameters from the histograms of the breakthrough forces of domains containing different amounts of cholesterol in high and low ionic strength buffer. Each histogram contained 200 measurements. The force presented is the central value of the Gaussian fit while the numbers in brackets correspond to the full width at half maximum (FWHM).

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