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Supplemental Information

Ordered Membrane Domain-Forming Properties of the Lipids of *Borrelia burgdorferi*

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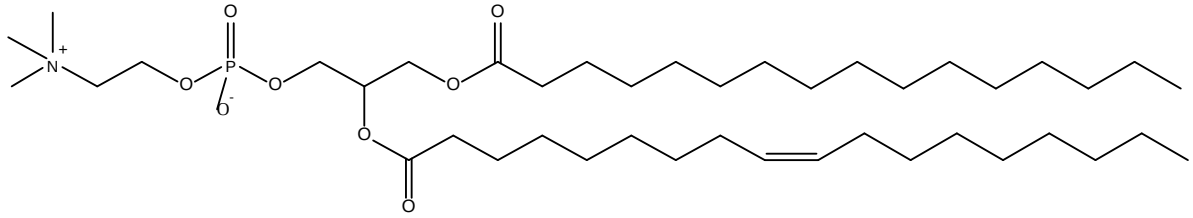
Supplemental materials for Z. Huang et al. "Ordered Membrane Domain Forming Properties of the Lipids of *Borrelia burgdorferi*"

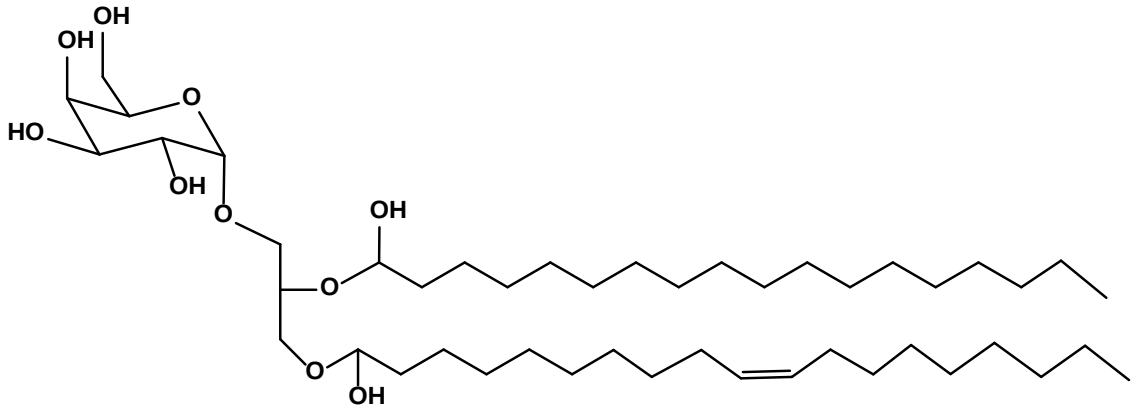
Supplemental Table 1. Vesicles size measurement by dynamic light scattering. Vesicles come from the samples used for FRET OR DPH fluorescence experiments discussed in the results. Measurements were taken at lipid concentration about 5-10 μM. The average from three measurements and standard deviations are shown. CHOL= cholesterol.

Lipids	**Average Radius (nm)**
***B.burgdorferi* 31 whole lipid extract**	59 ± 7
***B.hermsii* whole lipid extract**	66 ± 7
ACGal	325 ± 238
Bb.PC	67 ± 3
1:1 ACGal:Bb.PC	48 ± 12
1:1 ACGal:MGalD	837 ± 250
1:1 ACGal:DOPC	67 ± 9
1:1 CHOL:Bb.PC	60 ± 3
1:1 CHOL:MGalD	108 ± 49
1:1:1 ACGal:MGalD:Bb.PC	67 ± 11
1:1:1 ACGal:MGalD:DOPC	77 ± 12
1:1:1 CHOL:MGalD:Bb.PC	51 ± 10

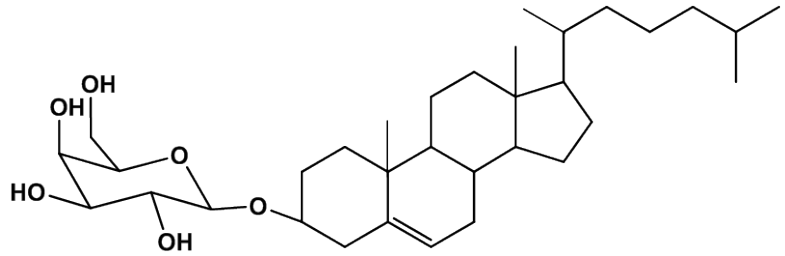
***Borrelia* PC (Bb.PC)**



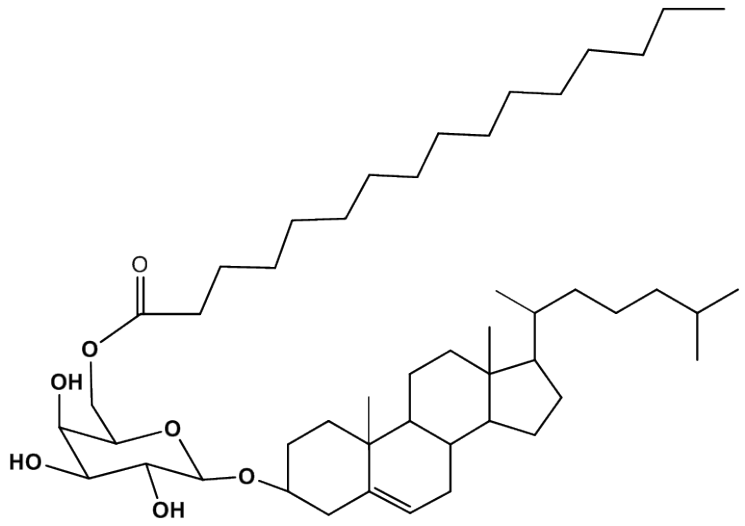
Mono-α-D-galactosyl-diacyl-glycerol (MGalD)



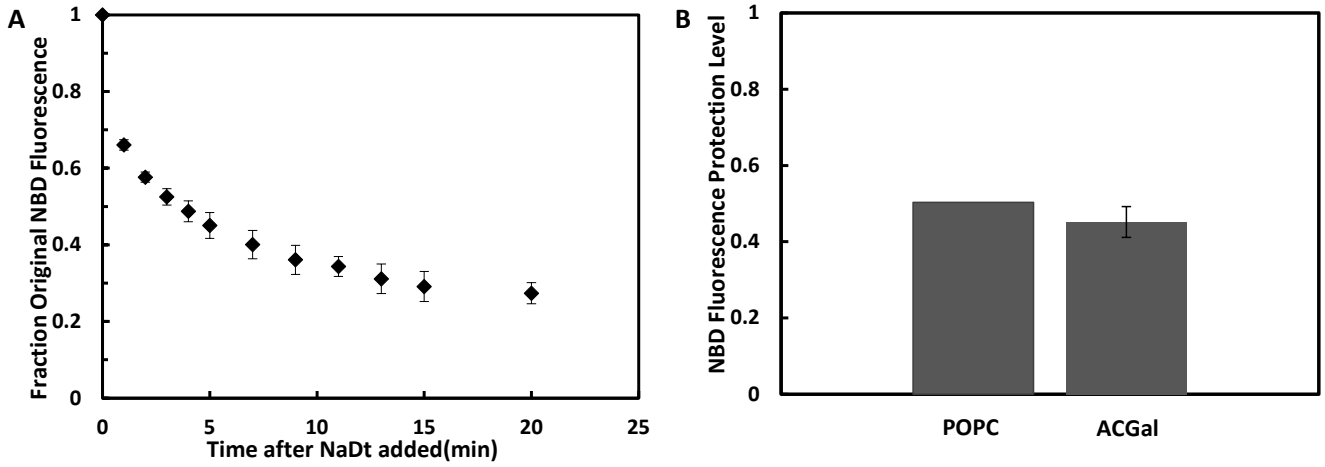
Cholesteryl-β-D-galactopyranoside (CGal)



Cholesteryl 6-*O*-acyl-β-D-galactopyranoside (ACGal)



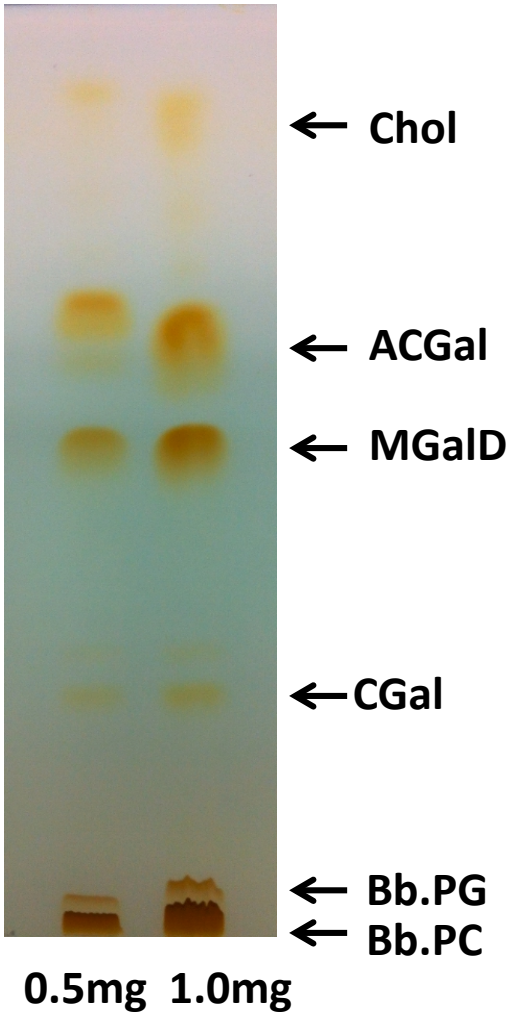
Supplemental Figure 1. B. burgdorferi individual chemical structure. The chemical structure of Bb.PC, MGalD, CGal and ACGal have been shown.



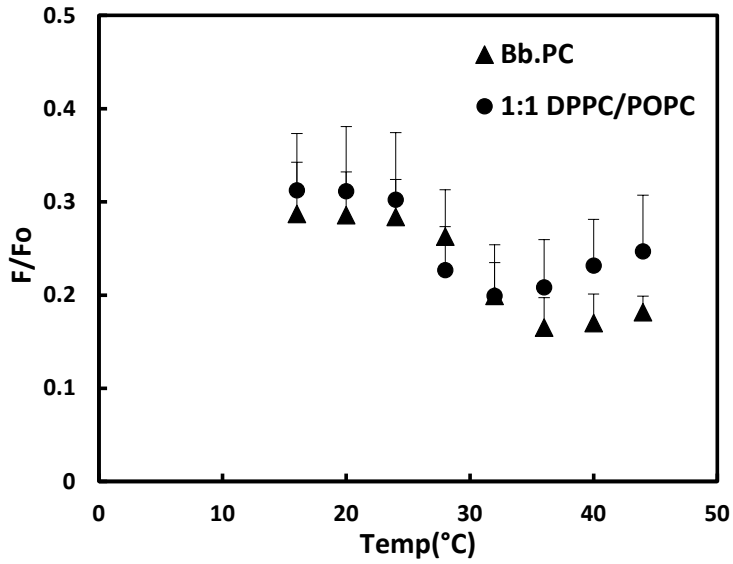
Supplemental Figure 2. Assay for impermeable vesicle formation by measuring the fraction of NBD-DOPE protection from externally added sodium dithionite. To prepare samples 0.1 mol% NBD-DOPE is co-dried with ACGal from organic solvent, and then suspended in PBS. After 10 mins sonication to disperse the lipids, sodium dithionite at a final concentration of 22mM NaDt (from 200 mM stock solution of sodium dithionite in 1M Tris buffer, pH~10) was added. Then NBD fluorescence (excitation wavelength 465 nm, emission wavelength 534 nm was measured versus time. A. The average fluorescence from three samples and standard deviation is shown. B. The extent of protection, which roughly gives the amount of lipid in the inner leaflet of any vesicles that formed. This is calculated by extrapolating the residual fluorescence after the initial reduction of outer leaflet NBD to time equals zero [1]. Notice that protection is close to 45%, as expected if the lipid is in the form of large vesicles with an intact bilayer. Similar behavior is seen for briefly sonicated POPC control vesicles. The protocol for POPC controls was similar to that for ACGal, except that a final concentration of 2 mM dithionite was used. The lower concentration of dithionite could be used because the reaction of NBD in the outer leaflet is much faster for POPC.

References

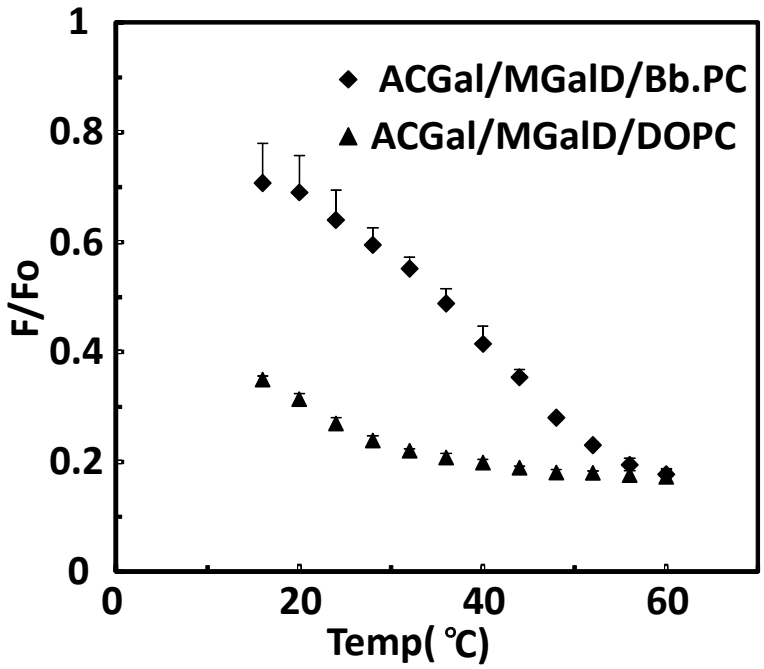
1. LeBarron, J. and E. London, *Effect of lipid composition and amino acid sequence upon transmembrane peptide-accelerated lipid transleaflet diffusion (flip-flop).* Biochim Biophys Acta (2016) **1858**, 1812-1820.



Supplemental Figure 3. Lipid profile of *Borrelia burgdorferi* B31 isolated on HP-TLC plates by CHCl3/methanol 85/15 (v:v). Total amount of loaded lipid shown at bottom of figure. The major lipids stained with iodine are: Bb.PC, Bb.PG, CGal, MGalD, ACGal, and cholesterol. Notice that Bb. PG and CGal are present are relatively low levels compared to ACGal, MGalD and Bb.PC.

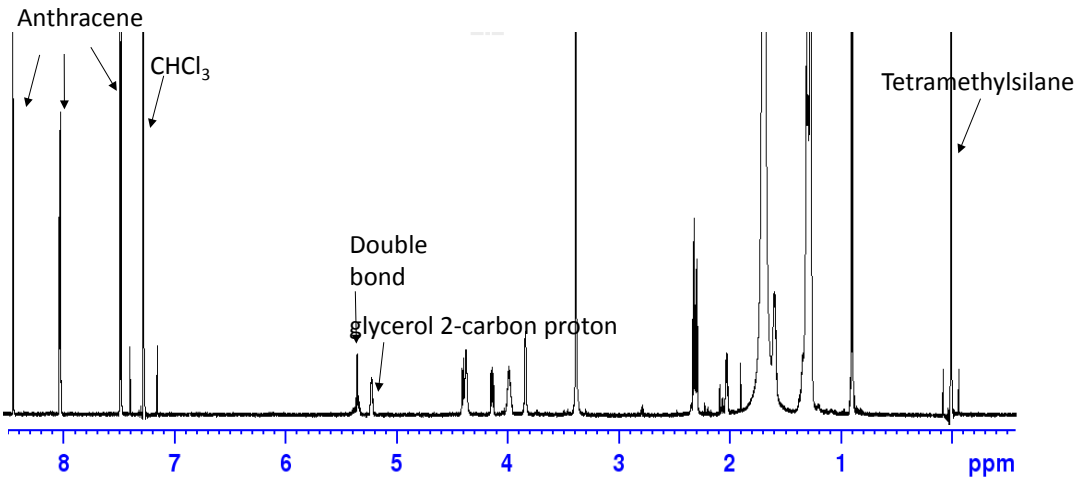


Supplemental Figure 4. Ability of pure Bb.PC ordered state domains in vesicles as assayed by FRET using DPH as FRET donor. (triangles) *B. burgdorferi* PC; (circles) 1:1 mol:mol DPPC/POPC (a mixture with similar acyl chain saturation level as Bb.PC). FRET donor: 0.1 mol% DPH; FRET acceptor 2 mol% rhod-DOPE.



Supplemental Figure 5. Comparison of ordered domain formation as a function of PC composition in vesicles containing ternary lipid mixtures with *Borrelia* lipids as detected by FRET. Ordered domain formation in vesicles composed of the ternary lipid mixture 1:1:1 (mol:mol) ACGal:MGalD:Bb.PC (diamonds) or 1:1:1 ACGal:MGalD:DOPC (triangles). Lipid concentrations and FRET measured as in Figure 3. The average from three samples and standard deviations are shown.

Bb.PC


Anthracene
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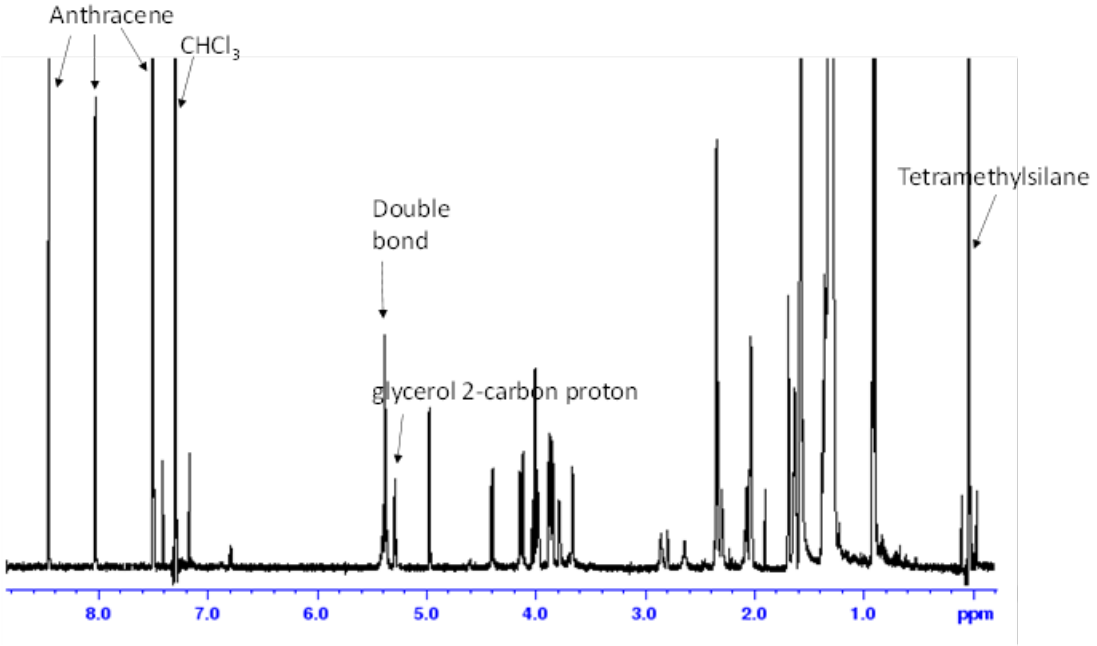
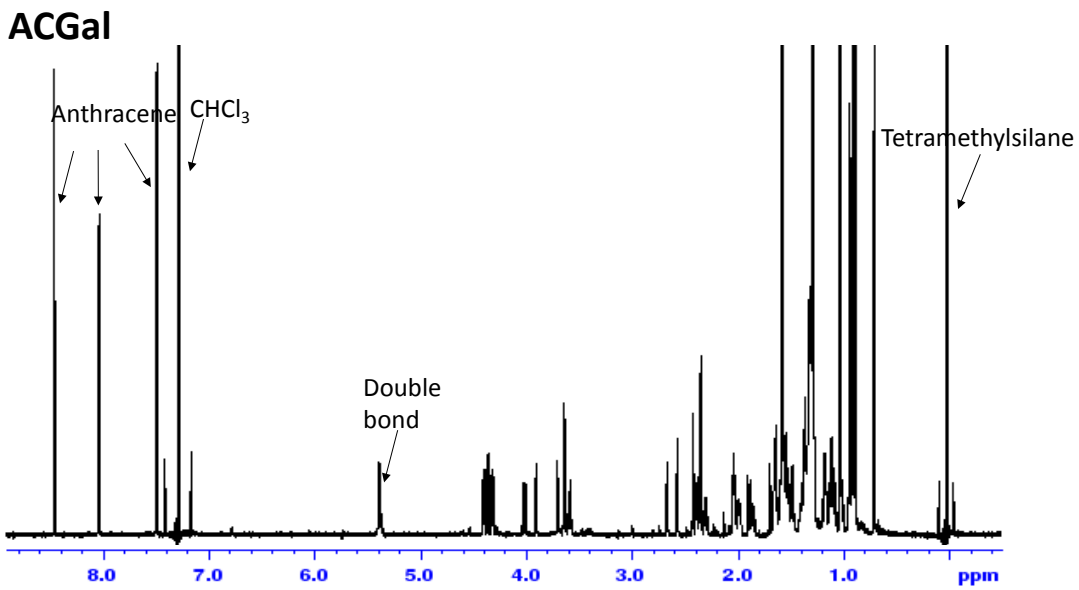
Tetramethylsilane
Double
bond
glycerol 2-carbon proton
8
7
6
5
4
3
2
1
ppm

MGalD


Anthracene
CHCl3
Tetramethylsilane
Double
bond
glycerol 2-carbon proton
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
ppm



Supplemental Figure 6. ^{1}H NMR spectra of *Borrelia* lipids. Top, Bb.PC; Middle, MGalD; Bottom, ACGal. 648 µM anthracene was used as an internal standard. Chemical shift at about 8.4ppm represents two anthracene protons. Acyl chain double bond vinyl proton signal is at 5.4 ppm and glycerol 2-carbon signal in Bb.PC and MGalD is at 5.3 ppm. Ratio of vinyl proton intensity to that of the proton on the 2-carbon of glycerol was used to determine double bond content for Bb.PC and MGalD using the following formula. Fraction of acyl chains with double bonds = 0.25 x (acyl chain vinyl proton intensity/glycerol 2 carbon intensity), where intensity is given by peak area. This gives a double bond/acyl chain ratio of 0.26 in Bb.PC and 0.75 in MGalD. For ACGal, the concentration of vinyl protons/concentration of ACGal molecules = (total vinyl proton peak intensity/intensity per proton), where the intensity per proton equals 0.5 x intensity of anthracene peak at 8.4 ppm (which comes from 2 protons) normalized to ACGal concentration, which was ~752 µM. The number of acyl chain double bonds per ACGal is then = (vinyl protons per ACGal - 1)/2. (The subtraction of one is to remove the contribution of the vinyl proton of cholesterol.) This is equivalent to 32% of ACGal acyl chains having one double bond.