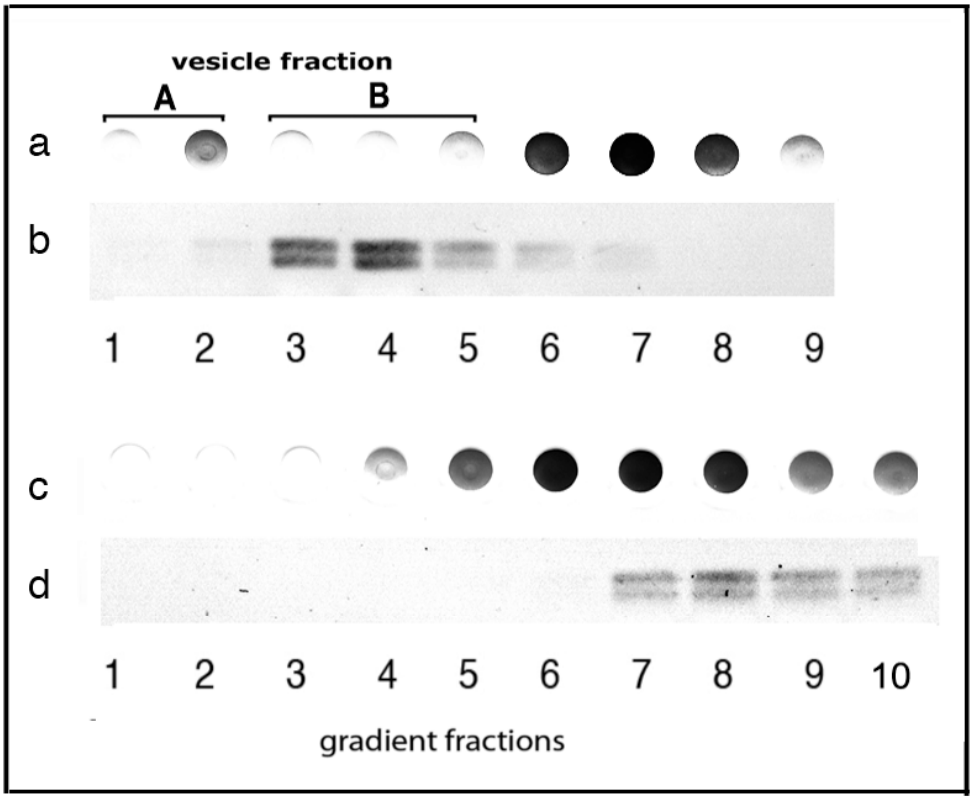
Supplemental Material

fig 1



Effect of temperature and detergent on gradient separation of VT1 binding vero cell derived vesicles. a) VT1 binding to vero cell DRM vesicle fractions separated by sucrose gradient centrifugation at 37°C (*1*), b) Gb_3 content of gradient fraction GSL extract determined by VT1/TLC overlay ; c) VT1 binding to vero cell membrane vesicles prepared by shear force(*2*) rather than detergent resistance, d) Gb_3 content of gradient fraction GSL extract determined by VT1/TLC overlay. The discord between VT1 vesicle binding and Gb_3 vesicle content is maintained at physiological temperature. VT1 bound only to the lighter vesicle fraction A, while Gb_3 is most prevalent in VT1-unbound vesicle fraction B, when DRM vesicles were separated at 37°C rather than 4 °C (a,b). Detergent soluble Gb_3 would explain VT1 binding to the more dense, unresolved fractions at the bottom of the gradient. In the absence of detergent (c,d), GSL containing gradient fractions were less well resolved in the gradient, but the discord between VT1 vesicle binding and Gb_3 vesicle content was maintained since VT1 bound fractions (5,6) containing little Gb_3. Thus this discord is not detergent induced.

Supplementary Table 1

Fatty acid content of Gb_3 within sucrose gradient separated vero cell derived vesicles determined by HPLC/tandem mass spectrometry.

	Control vero cells (% total Gb_3 in fraction)		MCD treated vero cells (% total Gb_3 in fraction)		Vero cells
Gb_3 fatty acid	Vesicle fraction A	Vesicle fraction B	Vesicle fraction A	Vesicle fraction B	membrane Gb_3
C24:0	33.8	31.1	37.2	30.5	30.4
C24:1	23.0	29.4	22.7	26.0	36.2
C22:0	20.8	19.9	17.2	17.1	9.0
C22:1	4.3	5.1	1.9	4.8	4.3
C20:0	1.2	1.4	2.0	0.8	0.5
C18:0	2.6	3.9	6.0	6.0	3.5
C16:0	14.2	8.5	11.8	13.3	15.5

Gb_3 within vesicle fraction A is bound by VT1 whereas vesicle B Gb_3 is refractory. VT1 vesicle B binding is increased after MCD cholesterol extraction. The Gb_3 fatty acid content is similar for fraction A and B vesicles with C24 predominating. The ratio of C24:1/C24:0 is higher in vesicle fraction B. MCD does not affect the fatty acid distribution profile.
Method: N-Palmitoyl-D3-lactosylceramide was used as an internal standard. Samples, calibrator or QC were extracted with 3 mL of chloroform/methanol, 2:1 (v/v). The sample was re-dissolved in methanol and analysed by LC-MS/MS. The chromatographic separation was achieved isocratically with methanol on an Agilent Eclipse XDB C8 column. Electrospray ionisation was conducted in positive mode on a Quattro Ultima mass spectrometer equipped with a Z-Spray ion source. Individual Gb_3 isoforms and the internal standard were detected using the multiple reaction monitoring (MRM) function.

Although the VT1 binding Gb_3 fraction(fraction A) can be separated from the bulk Gb_3 containing vesicles (fraction B) under our conditions, these vesicles colocalize with the bulk GSL containing fractions in the larger scale sucrose gradient separation of DRMs (i.e. in 'fraction 5') used previously(*3 , 4*). Centrifugation efficiency is an inverse function of the k factor $=2.53\text{x}10^5\text{x}\ \ln(r_{max}/r_{min})/(\text{rpm}/1000)^2$

(www.beckmancoulter.com/literature/Bioresearch/0828(DS).pdf). For constant r_{min}, k increases with tube length, explaining the increased resolution we observed.

references

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