

SUPPLEMENTARY REFERENCES

Chen, P.S., Toribara, T.Y., and Warner, H. (1956). Microdetermination of Phosphorus. *Analytical Chemistry* 28, 1756-1758.

Dorovska-Taran, V., Wick, R., and Walde, P. (1996). A ^1H nuclear magnetic resonance method for investigating the phospholipase D-catalyzed hydrolysis of phosphatidylcholine in liposomes. *Anal Biochem* 240, 37-47.

Jones, M.N. (2005). Use of liposomes to deliver bactericides to bacterial biofilms. *Methods Enzymol* 391, 211-228.

SUPPLEMENTARY FIGURE LEGENDS

Figure S1. Residual amounts of β -octyl-glucoside in liposome preparations.

(A) There is no significant difference in the levels of residual detergent between protein-free liposomes and SNAREs/Rab bearing proteoliposomes after dialysis. The recovered ^{14}C -activity (relative to the recovered lipid concentration) was correlated with the initial detergent:lipid ratio of 10:1 (before dialysis) to estimate the final detergent:lipid ratio. Shown is the average (\pm standard deviation) for $n=3$ independent preparations. The p -value was determined by a two-tailed independent two-sample t -test. (B) Almost all the detergent is dialyzed out during the initial dialysis steps. The ^{14}C -activity that was recovered after each step in the dialysis buffer (columns 2-7) is shown relative to the initially employed activity (column 1; $\sim 30 \mu\text{Ci}$ per reconstitution). Only about 0.01% of the initially added detergent is recovered with the final liposome suspensions (column 8). Shown is the average (\pm standard deviation) for $n=3$ independent preparations.

Figure S2. Characterization of proteoliposomes prepared for content-mixing assays.

(A) Lipid and protein recovery during RPL formation. Shown are the average values \pm standard deviations for three independent RPL preparations. (B) Size distribution of 4-

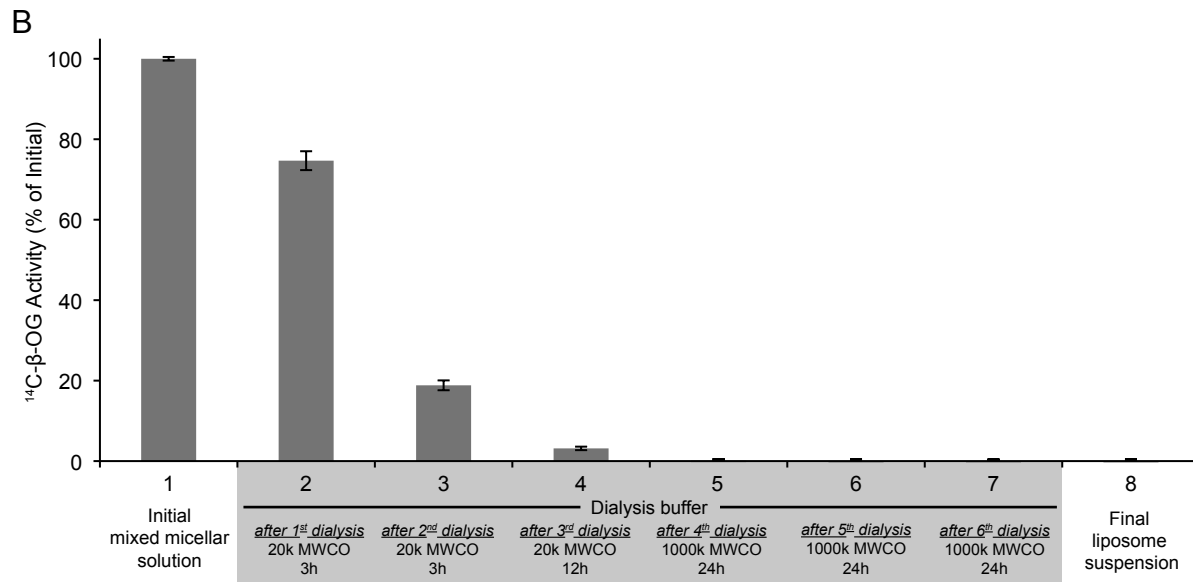
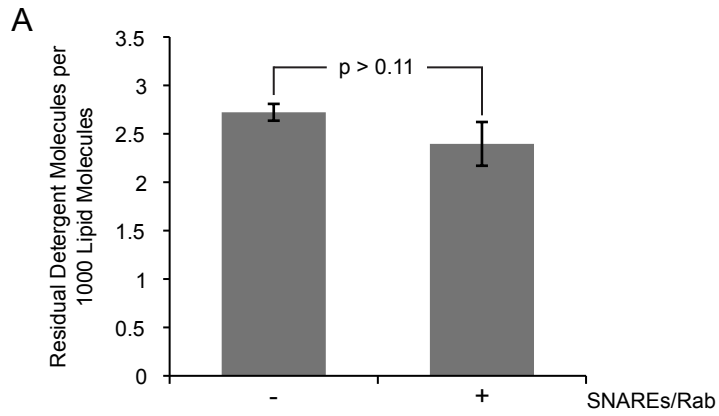
SNARE/Rab RPL population of vacuolar lipid composition. Representative example of size distribution (depicted as intensity particle size distribution) as obtained by dynamic light scattering. The average mean liposome size for 3 independent RPL preparations was 171.3 (\pm 13.4) nm. (C) Approximation of the number of entrapped molecules per RPL. The average number of entrapped molecules per 100,000 lipid molecules was determined as 3.67 (\pm 0.40) for R-phycoerythrin and 4.60 (\pm 1.63) for streptavidin-Cy5, respectively (n=6; involving 3 independent RPL preparations). As the number of entrapped molecules per RPL is dependent on the liposome size, these ratios have been related to the number of lipid molecules per RPL relative to their size (see supplementary materials and methods).

Figure S3. Neither fusion nor lysis can be observed for liposomes that do not bear SNARE and Rab proteins. Protein free liposomes (donors and acceptors, 250 μ M lipid each) were mixed in RB150 with 1 mM Mg²⁺/ATP, and incubated for 60 minutes at 27°C with either Sec17p/Sec18p/HOPS (squares), their respective buffers (circles), or detergent (diamonds). The means of the resulting PhycoE:Cy5 FRET signals of a triplicate assay are displayed with standard deviation, as percentages of the maximal values obtained by incubation with detergent.

Figure S4. A direct lysis-only-assay shows a cumulative lysis signal from consecutive events. Shown are typical examples of reactions that either measure the initial events of fusion or lysis (A; as in Fig.6), or a cumulative lysis signal only (B). These reactions have been run in parallel, and are representative for n=4 repeats. (A) RPLs (Sa-Cy5 bearing

donors and PhycoE-biotin bearing acceptors, 250 μM lipid each) were mixed in RB150 with $\text{Mg}^{2+}/\text{ATP}$, and incubated at 27°C in the presence (triangles) or absence (circles) of external dextran-biotin 70,000 (1 μM biotin) with either Sec17p/Sec18p/HOPS (filled circles/triangles), their respective buffers (open circles/triangles), or detergent (open diamonds for + biotin; - biotin not displayed). The resulting PhycoE:Cy5 FRET signals are displayed as percentages of the maximal values obtained by incubation with detergent in the absence of external biotin. (B) PhycoE-biotin bearing RPLs (500 μM lipid) were mixed in RB150 with 1 mM $\text{Mg}^{2+}/\text{ATP}$ and 16 nM Sa-Cy5, and incubated for 60 minutes at 27°C with either Sec17p/Sec18p/HOPS (filled circles/triangles), their respective buffers (open circles/triangles), or detergent (open diamonds for + biotin; - biotin not displayed). The resulting PhycoE:Cy5 FRET signals are displayed as percentages of the maximal values obtained by incubation with detergent in the absence of external biotin.

Zucchi & Zick 2011 - Supplementary Fig.S1

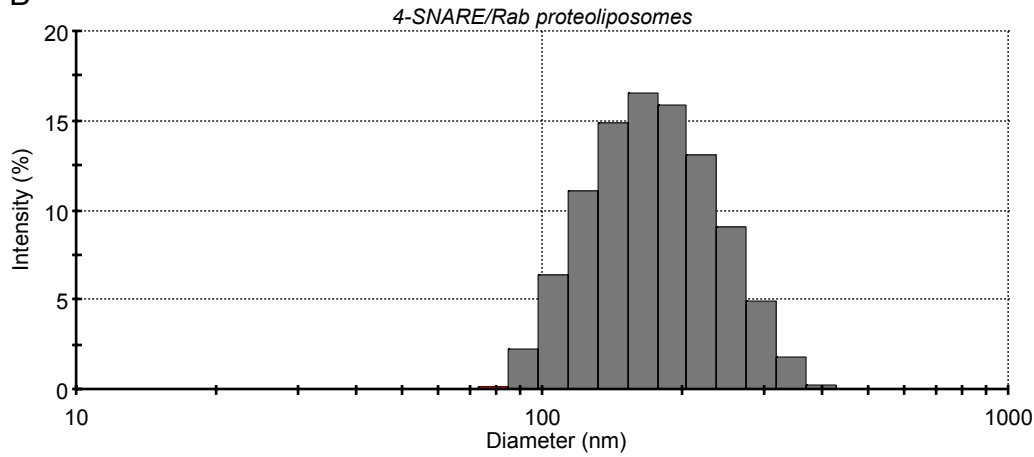


Zucchi & Zick 2011 - Supplementary Fig.S2

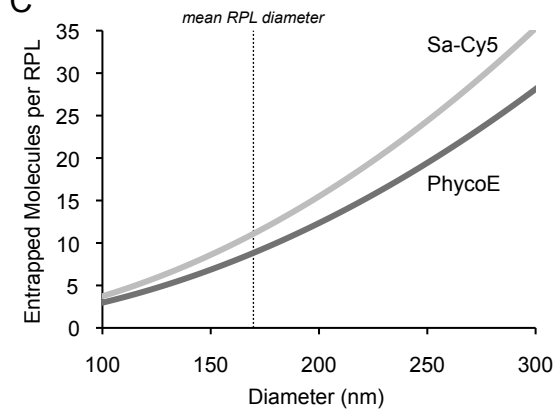
A

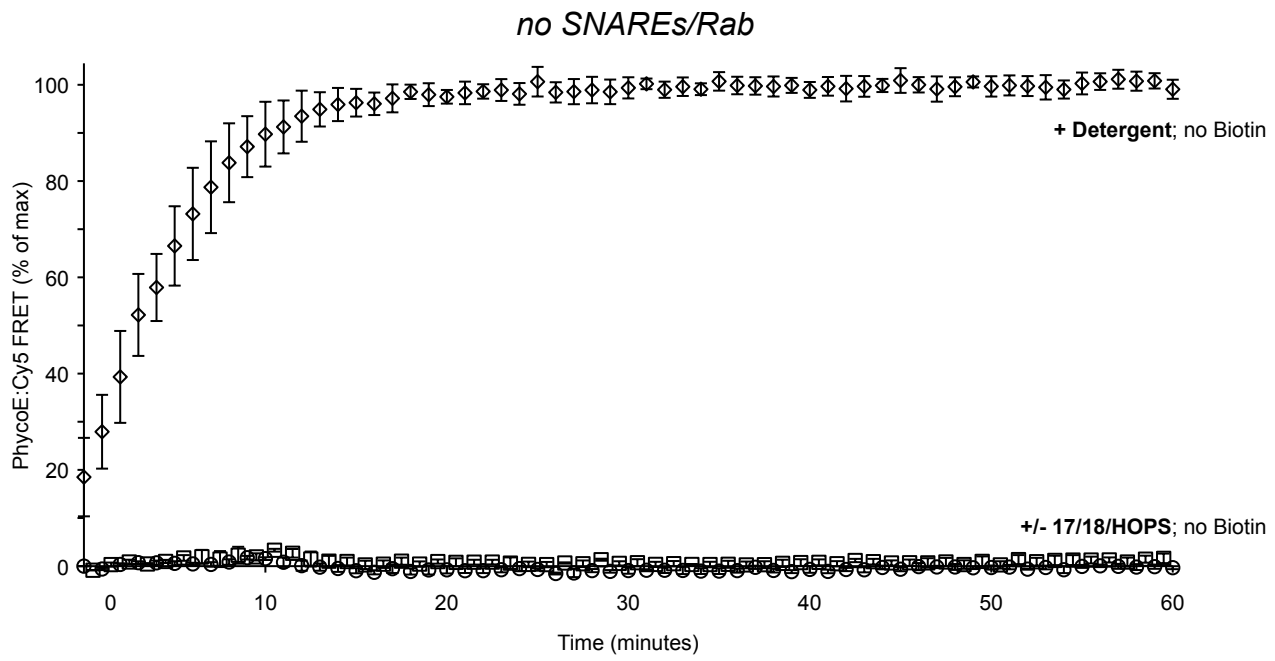
Lipids	<i>initial protein:lipid ratio</i>	<i>initial conc.</i>	<i>final conc.</i>	<i>% recovered</i>	<i>final protein:lipid ratio (average)</i>
		4 mM	1.95 ± 0.28 mM	62.5 ± 8.9	
Vam3p	1:1000	4 μM	1.57 ± 0.38 μM	50.2 ± 12.0	~ 1:1245
Vam7p	1:1000	4 μM	1.74 ± 0.20 μM	55.8 ± 6.3	~ 1:1120
Vti1p	1:1000	4 μM	2.38 ± 0.29 μM	76.2 ± 9.3	~ 1:820
Nvy1p	1:1000	4 μM	2.06 ± 0.15 μM	65.8 ± 4.7	~ 1:950
Ypt7p	1:10000	400 nM	230 ± 34 nM	73.7 ± 10.8	~ 1:8475

B

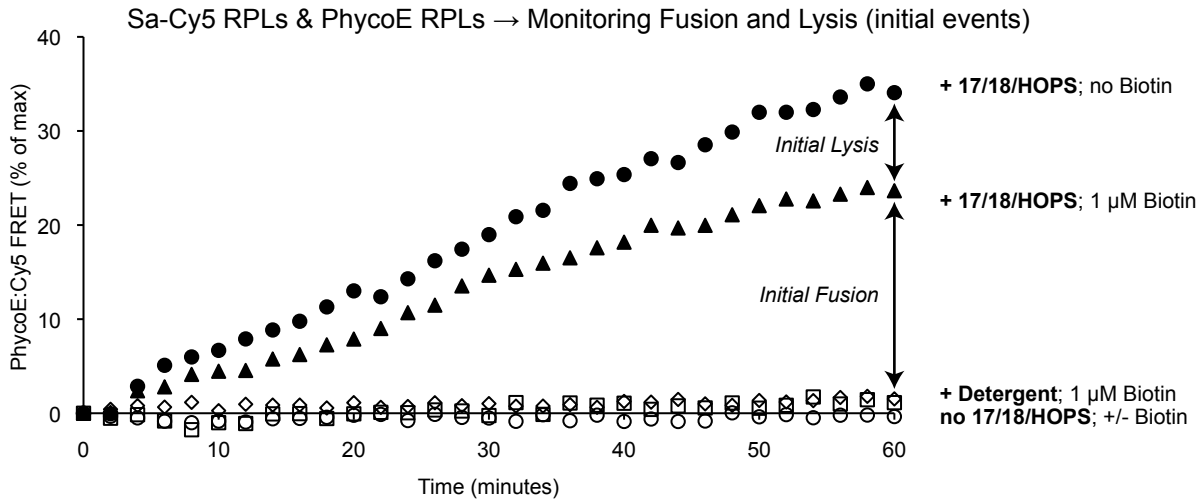


C





A



B

