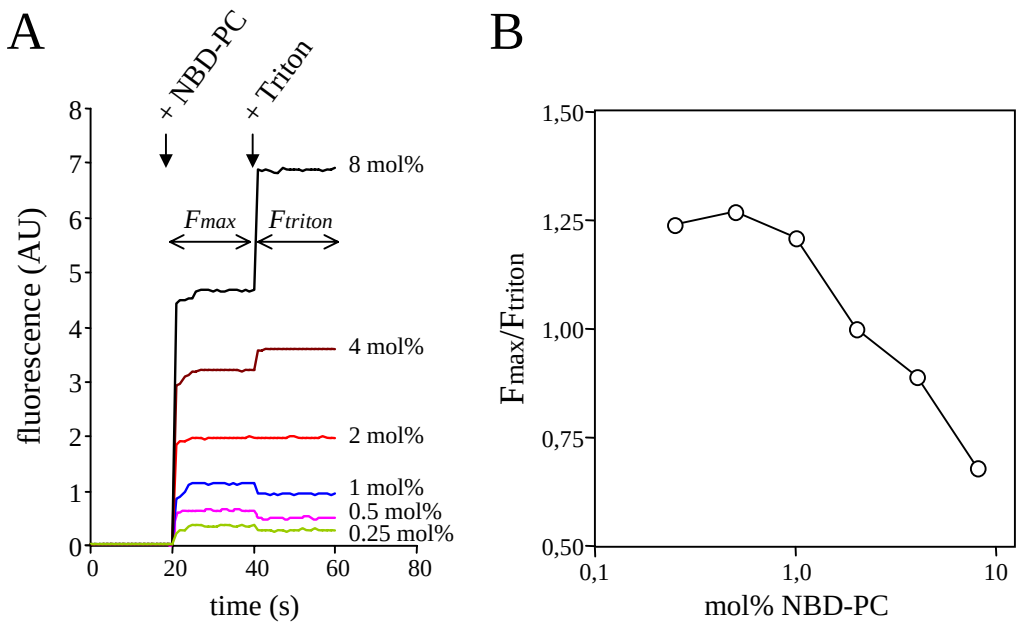
A
× NBD-PC
× Triton
8 mol%
Fmax
Ftriton
4 mol%
2 mol%
1 mol%
0.5 mol%
0.25 mol%
fluorescence (AU)
time (s)
0
1
2
3
4
5
6
7
8
0
20
40
60
80
B
Fmax/Ftriton
mol% NBD-PC
0,50
0,75
1,00
1,25
1,50
0,1
1,0
10

Supplementary Figure 1. Incorporation and quenching of NBD-PC in SVs. (A) Membranes enriched in SVs were prepared from 38°C-shifted *sec6-4* cells as described in Materials and Methods. The membrane suspension (1.5 μmol phospholipid/ml) was incubated at 25°C, and the fluorescence intensity was recorded at 540 nm (excitation at 480 nm, slit widths 4 nm, resolution 1 s). At t=20 s, NBD-PC was added at the given concentration (in mol% of the total phospholipid content) and the kinetics of membrane incorporation of the analogue was monitored by measuring the fluorescence intensity. Upon addition of NBD-PC, the fluorescence rapidly increased and reached a stable plateau (F_{max}). Addition of NBD-PC into buffer without SVs did not result in a significant increase of fluorescence (data not shown), since under these conditions NBD-labeled phospholipids are organized mainly in micelles in which their fluorescence is self-quenched. At t=40 s, 0.5% Triton X-100 was added to dissolve the membrane and to obtain maximal analogue dilution (F_{triton}) measured. At analogue concentrations up to 1 mol%, the fluorescence of NBD-PC was higher in the membrane than in the presence of Triton X-100 due to the higher quantum yield of membrane-associated analogue. However, with analogue concentrations above 2 mol%, the fluorescence increased when Triton X-100, indicating self-quenching of the membrane-associated analogue. (B) Determination of the critical quenching concentration of NBD-PC in SV-enriched membranes. The Triton X100-normalized fluorescence intensity (F_{max}/F_{triton}) was plotted against analogue concentration (mol%). At NBD-PC concentrations above 1 mol%, self-quenching of the analogue occurred as indicated by a gradual decrease in the reduction Triton X100-normalized fluorescence intensity. Consequently, for all lipid transport measurements, SV-enriched membranes were labeled with NBD lipids at a final concentration of 0.6 mol% of total phospholipid content.