Supplementary Information

The dipole potential of the lipid membrane determines mechanism of action of ester-stabilized phosphonium ylides

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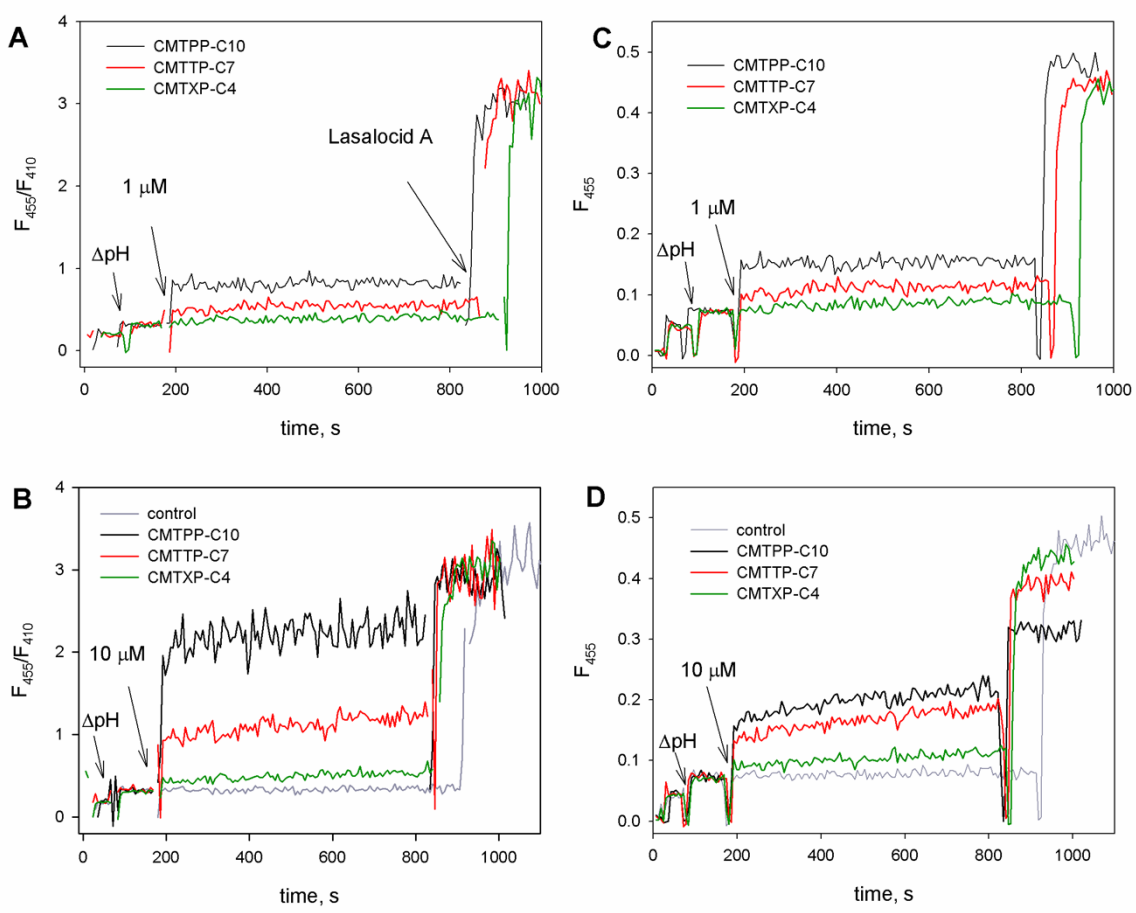

Fig. S1 Comparison of HPTS fluorescence kinetics measured at two excitation wavelengths. (**A, B**) HPTS fluorescence emission ratio F_{455}/F_{410} and (**C**, **D**) fluorescence emission F_{455} as a function of time after addition of phosphonium ylide precursors (1 μM – panels **A** and **C** or 10 μM – panels **B** and **D**). After t = 800 s, the lasalocid A was added to the cuvette to equilibrate the pH inside and outside the liposomes. The experiments were carried in the presence of 5 nM valinomycin and 1 mM p-xylene-bis-pyridinium bromide. Liposomes were from a mixture of POPC, POPG and cholesterol.

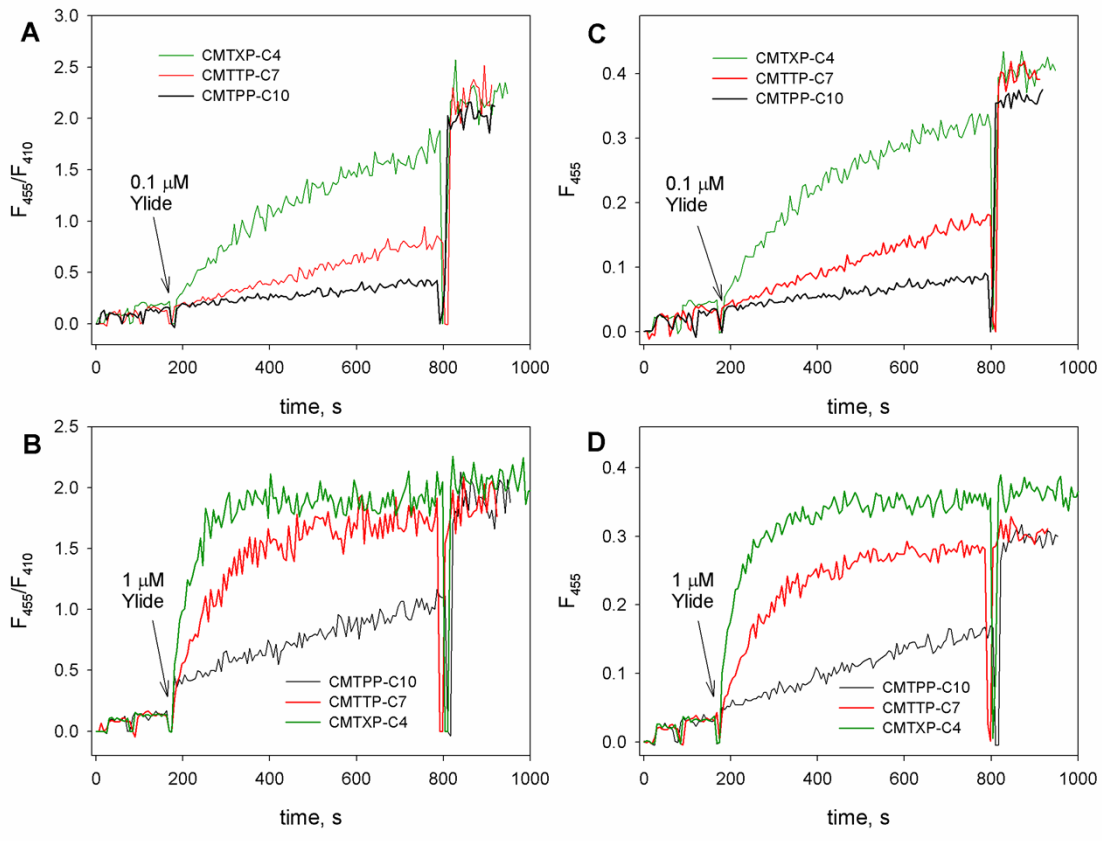

Fig. S2 Comparison of HPTS fluorescence kinetics measured at two excitation wavelengths. (**A**, **B**) HPTS fluorescence emission ratio F_{455}/F_{410} and (**C**, **D**) fluorescence emission F_{455} as a function of time after addition of phosphonium ylide precursors (0.1 μM – panels **A** and **C** or 1 μM – panels **B** and **D**). After t = 800 s, the lasalocid A was added to the cuvette to equilibrate the pH inside and outside the liposomes. The experiments were carried in the presence of 5 nM valinomycin and 1 mM p-xylene-bis-pyridinium bromide. Liposomes were from DPhytanylPC.

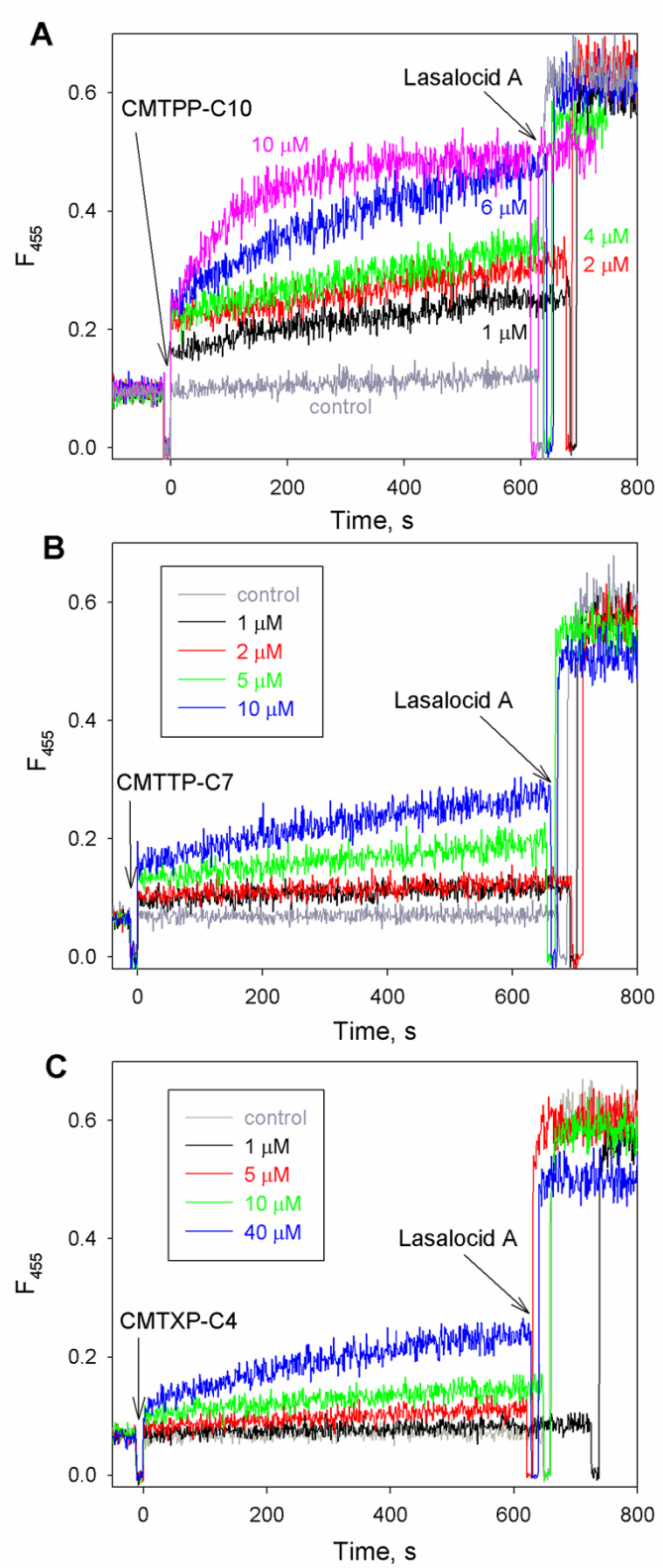

Fig. S3 Dissipation of the pH gradient by phosphonium ylide precursors on membranes of HPTS-loaded liposomes formed from POPC, POPG and cholesterol. The addition of CMTPP-C10 (panel **A**), CMTTP-C7 (panel **B**) or CMTXP-C4 (panel **C**) is indicated by an arrow. Concentrations of phosphonium salts are indicated on the panels. After t = 600 s, the lasalocid A was added to the cuvette to equilibrate the pH inside and outside the liposomes. The experiments were carried in the presence of 5 nM valinomycin and 1 mM p-xylene-bis-pyridinium bromide.

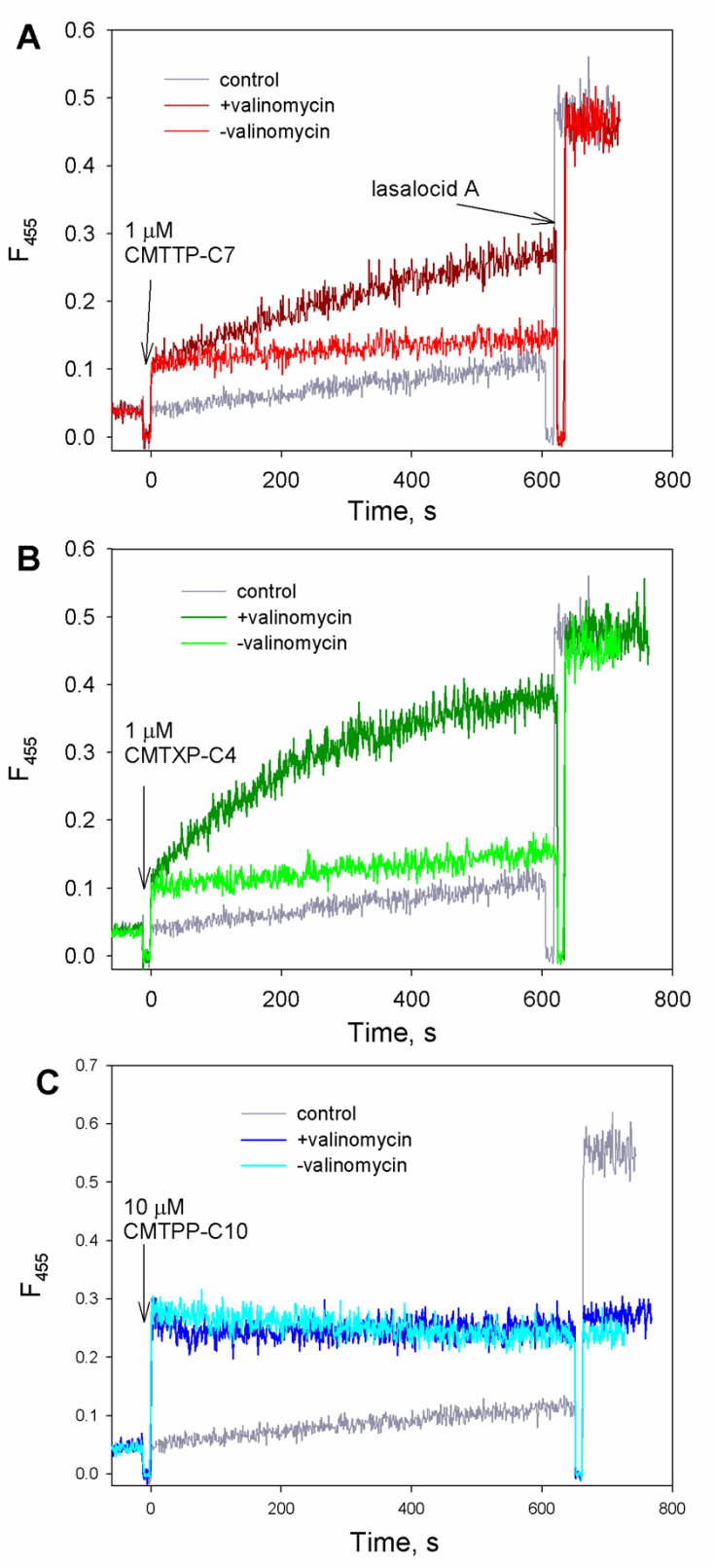

Fig. S4 Effect of valinomycin (5 nM) on the kinetics of HPTS fluorescence inside liposomes formed from DPhPC/PG. The addition of phosphonium salts is indicated by an arrow. The kinetics of HPTS fluorescence inside liposomes are shown for each ylide in different colors: CMTTP-C7 – red curves (panel **A**), CMTXP-C4 – green curves (panel **B**), CMTPP-C10 – blue curves (panel **C**) and control with adding ethanol – grey curve. After t = 600 s, the lasalocid A was added to the cuvette to equilibrate the pH inside and outside the liposomes. The experiments were carried in the presence of 1 mM p-xylene-bis-pyridinium bromide. Valinomycin did not affect the control fluorescence kinetics.

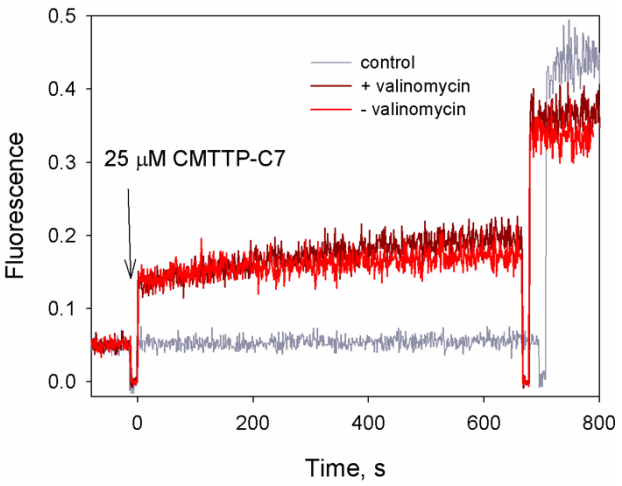

Fig. S5 Effect of valinomycin (5 nM) on the kinetics of HPTS fluorescence inside liposomes formed from POPC, POPG and cholesterol. The addition of 25 μM CMTTP-C7 is indicated by an arrow. After t = 600 s, the lasalocid A was added to the cuvette to equilibrate the pH inside and outside the liposomes. The experiments were carried in the presence of 1 mM p-xylene-bis-pyridinium bromide. Valinomycin did not affect the control fluorescence kinetics.

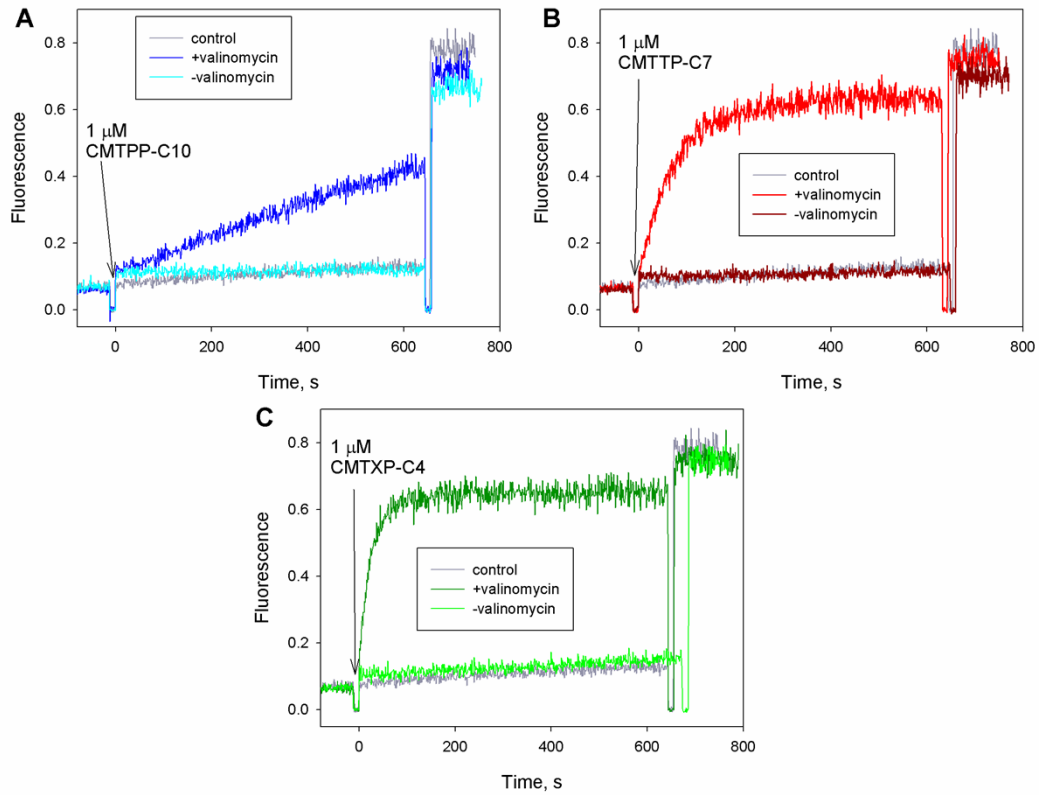

Fig. S6 Effect of valinomycin (5 nM) on the kinetics of HPTS fluorescence inside liposomes formed from DPhytanylPC. The addition of phosphonium salts is indicated by an arrow. The kinetics of HPTS fluorescence inside liposomes are shown for each phosphonium salt in different colors: CMTPP-C10 – blue curves (panel **A**), CMTTP-C7 – red curves (panel **B**), CMTXP-C4 – green curves (panel **C**) and control with adding ethanol – grey curve. After t = 600 s, the lasalocid A was added to the cuvette to equilibrate the pH inside and outside the liposomes. The experiments were carried in the presence of 1 mM p-xylene-bis-pyridinium bromide.

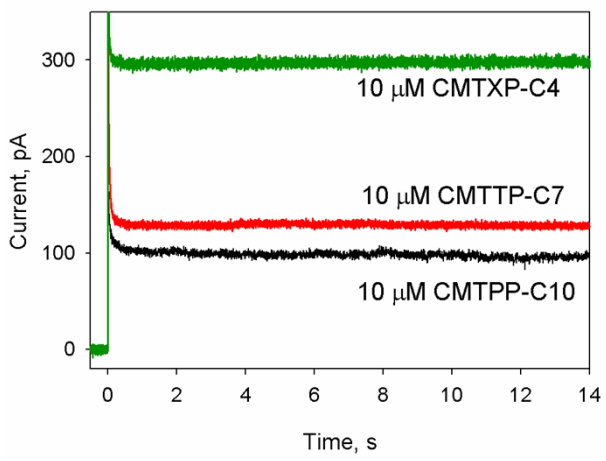

Fig. S7 Time courses of electric current through planar bilayer lipid membrane after a voltage jump from 0 mV to 50 mV at t=0 s in the presence of 10 μM CMTPP-C10 (black curve), CMTTP-C7 (red curve) and CMTXP-C4 (green curve). BLM was formed from DPhPC. The solution was 10 mM Tris, 100 mM KCl, pH=7.6.

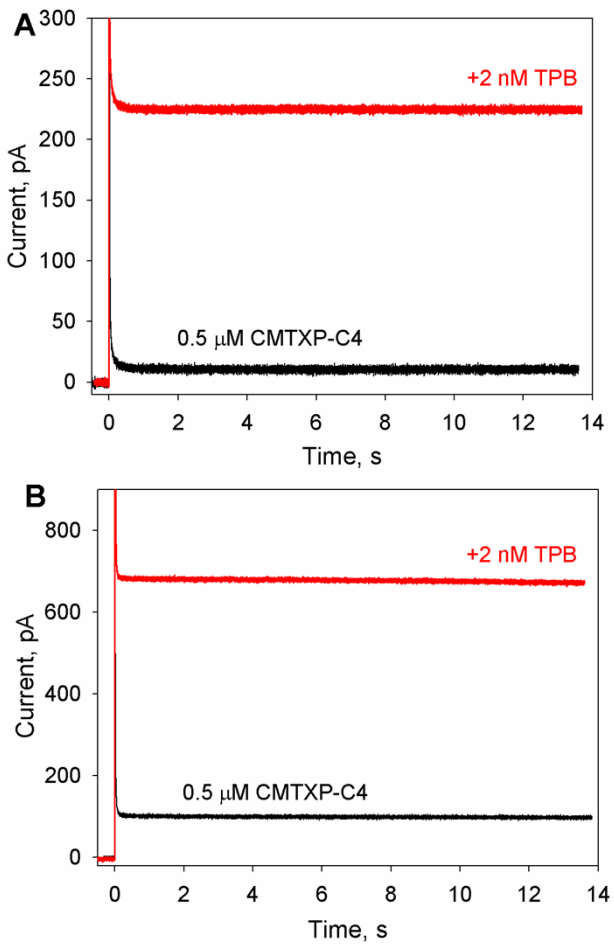

Fig. S8 Time courses of electric current through planar bilayer lipid membrane after a voltage jump from 0 mV to 50 mV at t=0 s in the presence of 0.5 μM CMTXP-C4 with (red curve) or without (black curve) addition of 2 nM TPB. BLM was formed from DPhPC (panel **A**) or from lipid mixture DPhPC/DPhPG (70/30, w/w) (panel **B**).

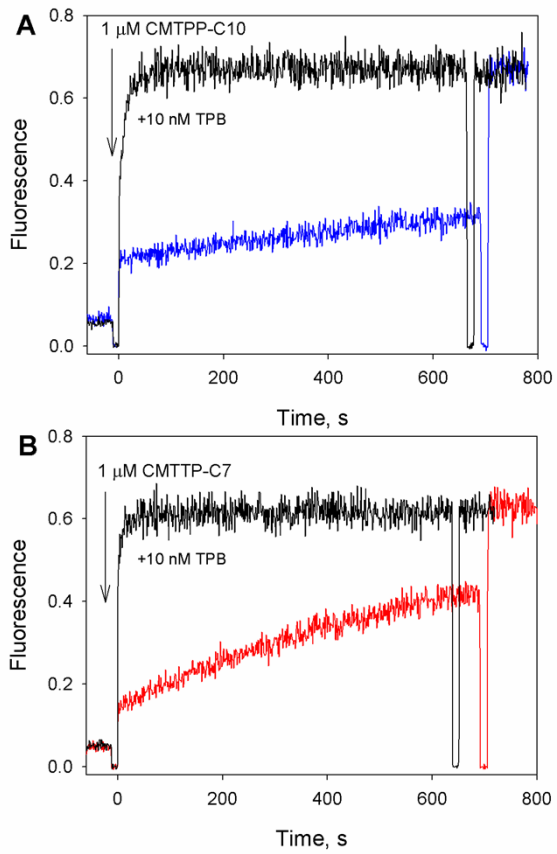

Fig. S9 Effect of TPB on dissipation of the pH gradient by CMTPP-C10 (panel **A**), CMTTP-C7 (panel **B**) on membranes of HPTS-loaded liposomes formed from DPhPC/PG. The addition of the phosphonium salts is indicated by an arrow. At the end of the recording the lasalocid A was added to the cuvette to equilibrate pH inside and outside the liposomes. The experiments were carried in the presence of 5 nM valinomycin and 1 mM p-xylene-bis-pyridinium bromide.

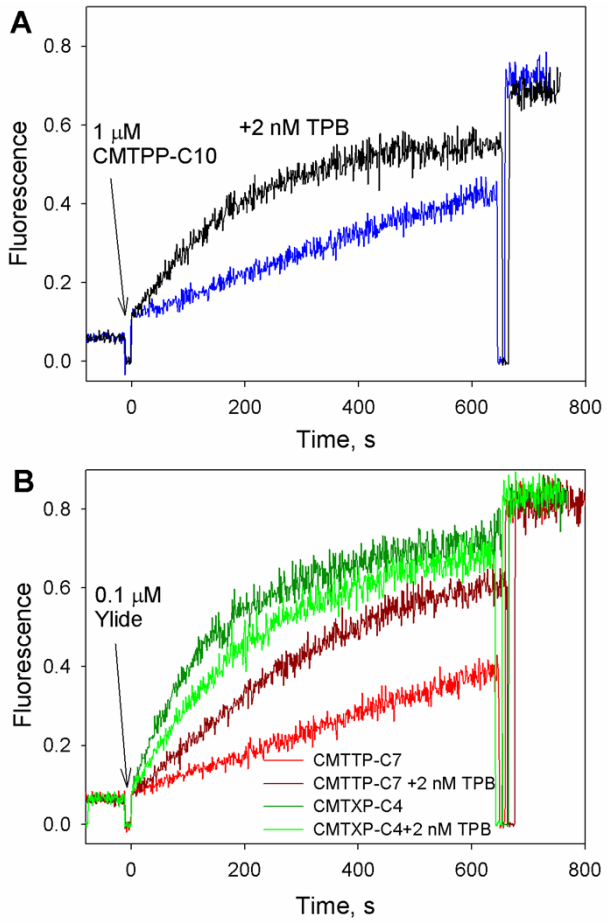

Fig. S10 Effect of TPB on dissipation of the pH gradient by CMTPP-C10 (panel **A**), CMTTP-C7 and CMTXP-C4 (panel **B**) on membranes of HPTS-loaded liposomes formed from DPhytanylPC. The addition of the phosphonium salts is indicated by an arrow. At the end of the recording the lasalocid A was added to the cuvette to equilibrate pH inside and outside the liposomes. The experiments were carried in the presence of 5 nM valinomycin and 1 mM p-xylene-bis-pyridinium bromide.

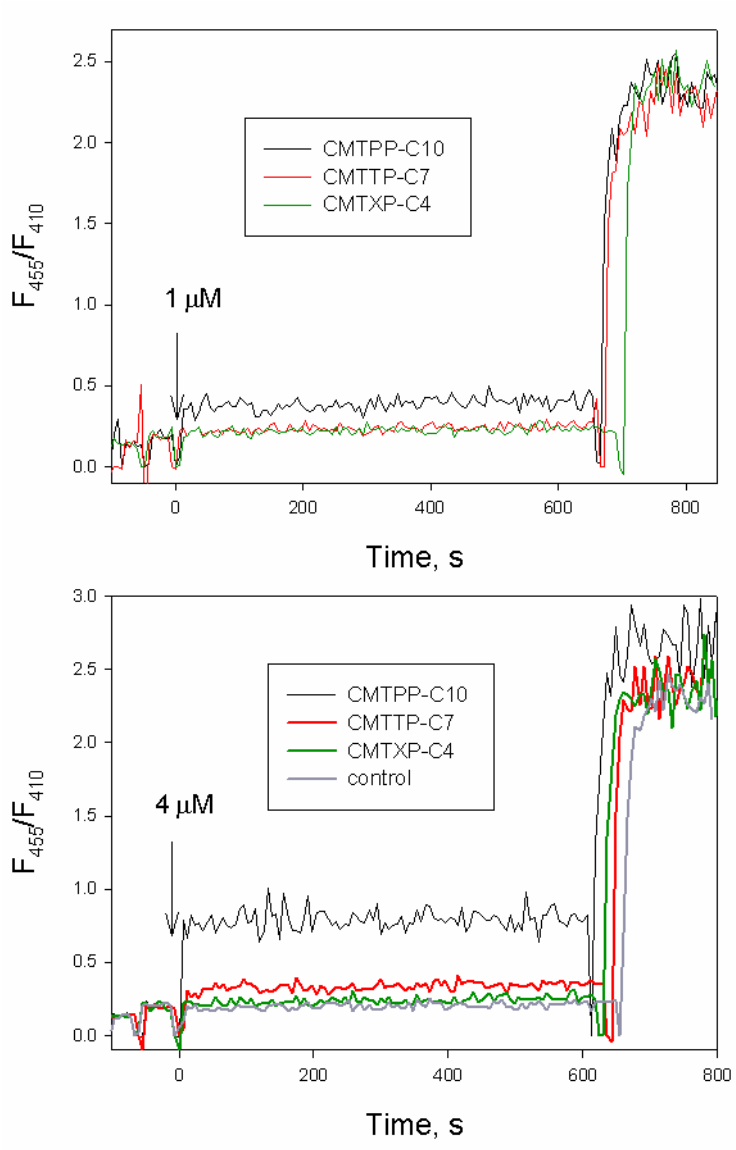

Fig. S11 HPTS fluorescence emission ratio F_{455}/F_{410} as a function of time after addition of phosphonium ylide precursors (1 μM or 4 μM). After t = 600 s, the lasalocid A was added to the cuvette to equilibrate the pH inside and outside the liposomes. The experiments were carried in the presence of 5 nM valinomycin and 1 mM p-xylene-bis-pyridinium bromide. Liposomes were from a mixture of POPC and cholesterol (70:30, w:w).

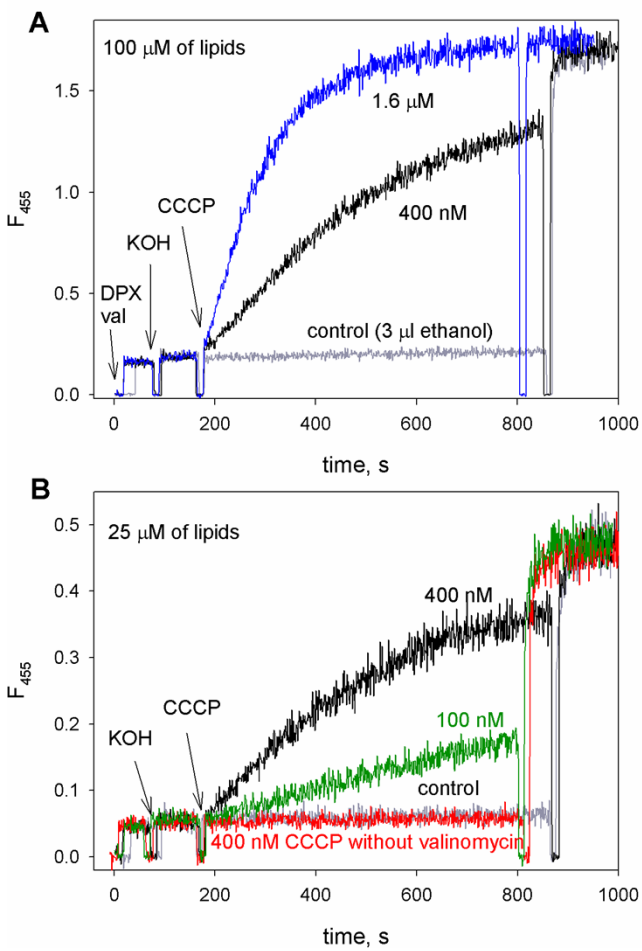

Fig. S12 The kinetics of HPTS fluorescence inside liposomes formed from POPC, POPG, cholesterol after addition of protonophore CCCP (0 nM – gray line, 400 nM –black line, 1,6 μM – blue line, 100 nM – green line, 400 nM without valinomycin – red line) depending on lipid concentration (100 μM – **A**, 25 μM – **B**). After t = 800 s, the lasalocid A was added to the cuvette to equilibrate the pH inside and outside the liposomes. The experiments were carried in the presence of 5 nM valinomycin and 1 mM p-xylene-bis-pyridinium bromide.