**Supplementary Materials**

# The role of lipids and cations in membrane fusion mediated by the heptad repeat domain 1 of Mitofusin

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**Figure S1**

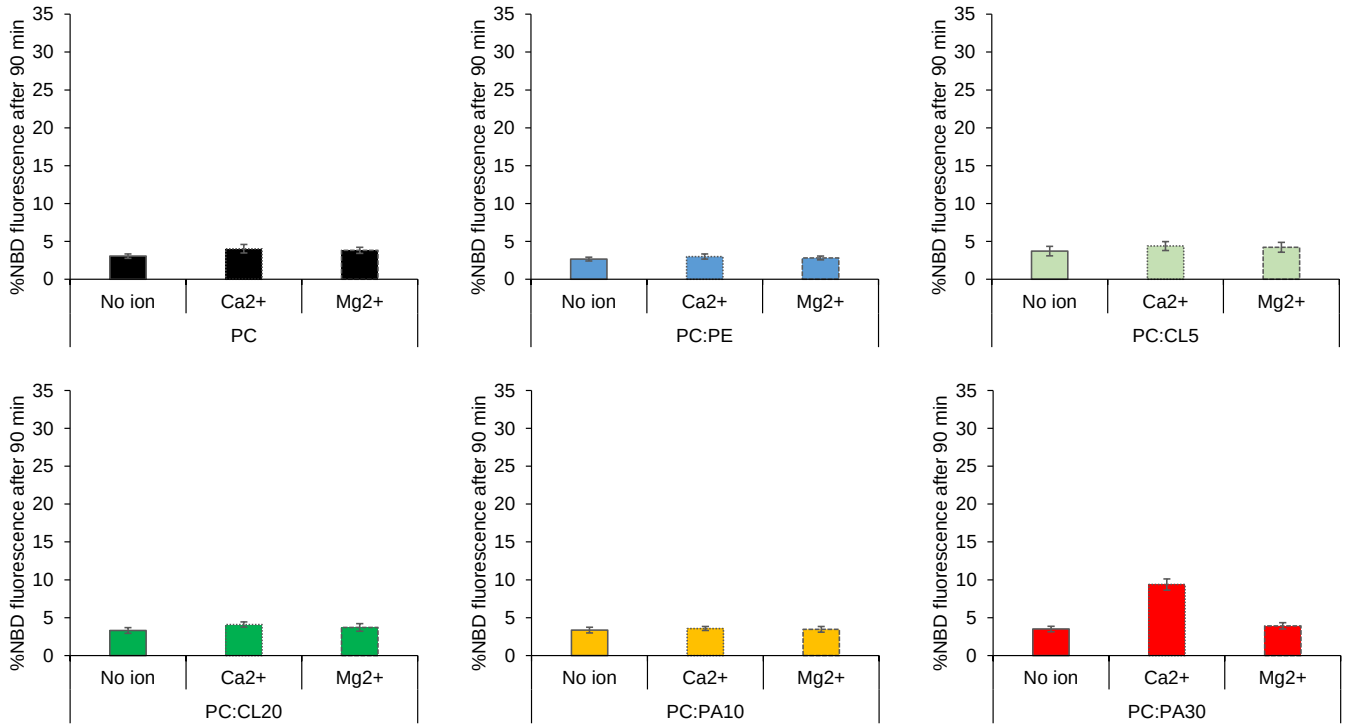


**Figure S1.** Average extent of lipid mixing after a 90-min reaction in fusion control experiments with liposomes functionalized with NTA-Ni lipids, where buffer alone was used instead of HR1. The experimental conditions are the same as those in Figures 1-4. Data represent the average of n=3-20 independent experiments, with error bars indicating standard errors of the mean.

**Figure S2**

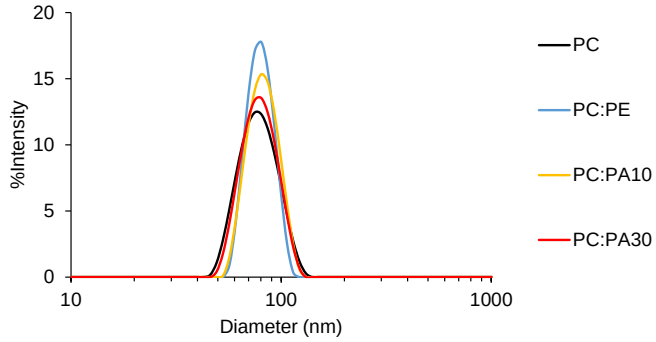


**Figure S2.** Representative size distribution of liposomes functionalized with NTA-Ni lipids, determined by multi-angle dynamic light scattering, as a function of their lipid composition. Lipid compositions are the same as those in Figures 1-4.

**Figure S3**

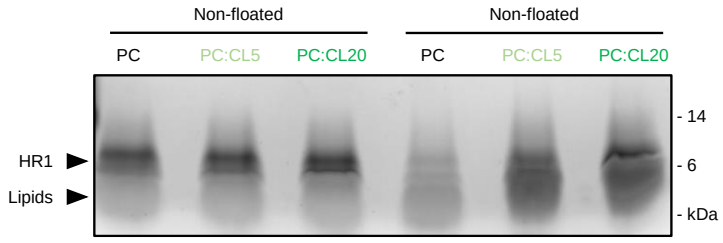


**Figure S3.** Liposomes with the same lipid compositions as in Figure 3 were incubated with HR1-$His_6$ peptides (500 μM of lipids and 25 μM of peptides) at 37°C for 1 hour. The reaction mixes were separated using a discontinuous nycodenz gradient to distinguish HR1-bound liposomes from unbound HR1. Protein and lipid recoveries in the floated samples are estimated by SDS-PAGE stained with Coomassie upon comparison with the non-floated samples.

**Figure S4**

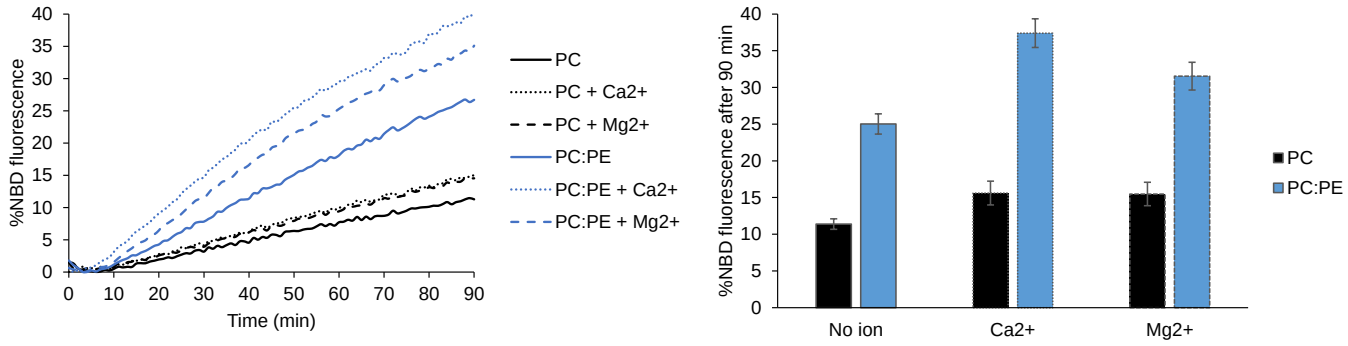


**Figure S4.** (**Left**) Representative kinetics of a FRET-based lipid mixing assay between liposomes containing 5 mol% NTA-Ni lipids in their membrane, along with 95 mol% PC (black) or 65 mol% PC and 30 mol% PE (blue). The fusion reaction was initiated by adding HR1-$His_6$ peptides at t=0 in the absence or presence of the divalent cations $Ca^{2+}$ or $Mg^{2+}$ (500 μM of lipids, 25 μM of peptides, and 1 mM of cations). Control experiments with buffer alone instead of HR1 are presented in Figure S1; (**Right**) Average extent of lipid mixing observed after a 90-min period, based on data from n=6-21 independent experiments. The error bars represent the standard errors of the mean.