Supplementary information

Cooperativity between sodium ions and water molecules facilitates lipid mobility in model cell membranes

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Experimental procedures

Materials: 1,2-Dimyristoleoyl-sn-glycero-3-phosphocholine (14:1 PC), egg yolk sphingomyelin (SM), and cholesterol were purchased from Avanti Polar Lipids, Alabaster, AL, USA. 1,2-dioleoyl-snglycero-3-phosphoethanolamine labeled with Atto 633 (DOPE-Atto 633), 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES), sodium chloride (NaCl), calcium chloride ($CaCl_2$), Magnesium chloride ($MgCl_2$), potassium chloride (KCl), 1,2-dioleoyl-sn-glycero-3- phosphoethanolamine-N-(7-nitro-2−1,3-benzoxadiazol-4-yl)-ammonium salt (18:1 NBD DOPE), 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine-N-(7-nitro-2-1,3-benzoxadiazol-4-yl)-ammonium salt (16:0 NBD DPPE), TopFluor cholesterol and chloroform (HPLC grade) were purchased from Merck KGaA, Darmstadt, Germany. All the materials were used without further purification.

Vesicle Preparation: Vesicle deposition method following a formerly established protocol with suitable adaptations was used to prepare SLBs[1]. 14:1 PC, SM, and cholesterol were mixed at a molar ratio 1:1:1 with an appropriate fluorescent label (0.5 mol% of NBD-PE or 0.1 mol% of TopFluor cholesterol) in chloroform solution. The chloroform was evaporated by blowing nitrogen gas and to ensure complete removal of the solvent, the dried thin film of lipids deposited on the bottom of the vial was desiccated under vacuum for at least 2 h. The lipids were dissolved in an approapriate buffer solution (pH adjusted to 7.4) to form multilamellar vesicles (MLVs) of overall 10 mM lipid concentration and were heated to 60°C on a hot plate and vortexed for a few cycles to obtain a completely homogeneous aqueous solution of lipids. The MLV suspension was distributed as aliquots into sterilized glass vials and those were stored at −20 °C for further use.

Preparation of SLBs of varying salt concentration: HEPES buffers of varying Na^+ ion concentrations were prepared by adding suitable amounts of NaCl salt with HEPES in fresh Milli Q water. Buffer solutions containing 5 mM, 20 mM, 50 mM, 150 mM and 1.5 M NaCl with 10 mM HEPES were used to investigate the effect of sodium ions on SLBs. Additionally, fresh Milli Q water, 10 mM HEPES buffer, 150 mM NaCl solution in fresh Milli Q water as well as 10 mM HEPES buffer solutions with 150 mM $CaCl_2$, $MgCl_2$ and KCl were also used. The pH of the buffer solutions was around 5.2 naturally after disolving HEPES to fresh Milli Q water. The pH was not adjusted to the biological pH range (pH ~7.4) to refrain from altering the sodium ion concentration by adding excess NaOH. Another reason was to keep the pH comparable to the pH of Milli Q water, which came down to around 5.5 soon after the collection due to absorption of atmospheric CO_2. The frozen 10 mM MLV aliquots were thawed and diluted 10 times (final concentration of lipids 1 mM) using a suitable water/ buffer solution with desired salt concentration. These cloudy MLV suspensions were bath-sonicated for 10 mins to generate a clear solution of small unilamellar vesicles (SUVs). A half-cut Eppendorf tube was placed on a coverslip with a freshly cleaved mica sheet glued on it by UV-activated glue (Norland 68) and was sealed with silicone. At room temperature, 100 µL of SUVs solution in the desired buffer was deposited on the mica followed by immediate addition of 2 µL of 0.1 M $CaCl_2$ solution. 400 µL of Milli Q water or buffer solution of desired salt concentration was added to it after around 30 sec and then the sample was incubated for 30 minutes. The bilayer was rinsed with total of 20 mL of specific buffer solution to eliminate excess unburst vesicles. The Eppendorf tube reservoir was fully filled with desired buffer solution and sealed with a glass coverslip and silicone to prepare a fully hydrated sample.

Preparation of SLBs of different hydration states: The SLBs were dehydrated by the removal of bulk water/buffer solution using pipette and immediate purging with nitrogen gas using our home-built humidity control setup by the method of slow and gradual decrease of the relative humidity[2]. SLBs were equilibrated for about 10 mins to 85%, 70%, 50%, 30% and 3% RH before FRAP measurements were performed.

Fluorescence Microscopy and FRAP: Fluorescence imaging and FRAP experiments were performed on SLBs in buffer with varying salt concentrations using an upright Zeiss LSM 710 (Carl Zeiss, Jena, Germany) microscope with 100x, 1.3 NA oil immersion objective. Fluorescent dyes NBD-DOPE, NBD-DPPE and TopFluor cholesterol were excited by laser of wavelength 488 nm. Confocal imaging and FRAP experiments were performed on at least three samples of each salt concentration at fully hydrated condition to obtain the average domain size and diffusion coefficient. Minimum 10 different, 50x50 µm areas were imaged for each sample for domain size determination. The original confocal images were converted to 8-bit black and white binary images. Domains were identified by adjusting the threshold of contrast, and the domain sizes were obtained using the ImageJ software[3].

FRAP experiments were performed on at least 6 different, 50 µm × 50 µm areas of each hydrated and dehydrated sample by bleaching a circular spot of 10 µm diameter. Diffusion coefficients of lipids were quantified by fitting the fluorescence recovery curves assuming free Brownian lateral diffusion of lipid molecules in the membrane using modified Soumpasis formula[4]:

$$f(t) = a \cdot e^{\frac{2\tau_D}{t}} \left(I_0\left(\frac{2\tau_D}{t}\right) + I_1\left(\frac{2\tau_D}{t}\right) \right) \quad (1)$$

where

$$\tau_D = \frac{w^2}{4D} \quad (2)$$

where a is an amplitude of the fitted recovery curve, b is the fluorescence intensity remaining after photobleaching, w is the radius of the bleached area, and $I_0(t)$ and $I_1(t)$ are modified Bessel functions. Intensity of the bleached spot was normalized with respect to the reference intensity signal of the whole image excluding a circular area around the bleached spot with radius $2w$.

Results and Discussion

Supplementary Note 1

Preparation of SLBs in buffer conditions depleted in ions and control over their hydration state do not pose major difficulties. At the same time it should be noted that membrane preservation after dehydration appeared to be more difficult for SLBs with lower salt concentrations. Often, in large area of the sample, a dense layer of aggregates was observed on top of the membrane and membrane coverage of the solid support (mica) was lower. This suggests decreased mechanical stability of the membrane in the absence (or low content) of ions. This observation is consistent with previous atomic force microscopy, transmission IR and force spectroscopy studies, which showed that presence of ions leads to an increase of the mechanical strength of SLBs. This has been ascribed to the decrease in intermolecular distances due to screening of polar head groups and enhancement of the order of hydrocarbon tails in presence of various cations[5,6].

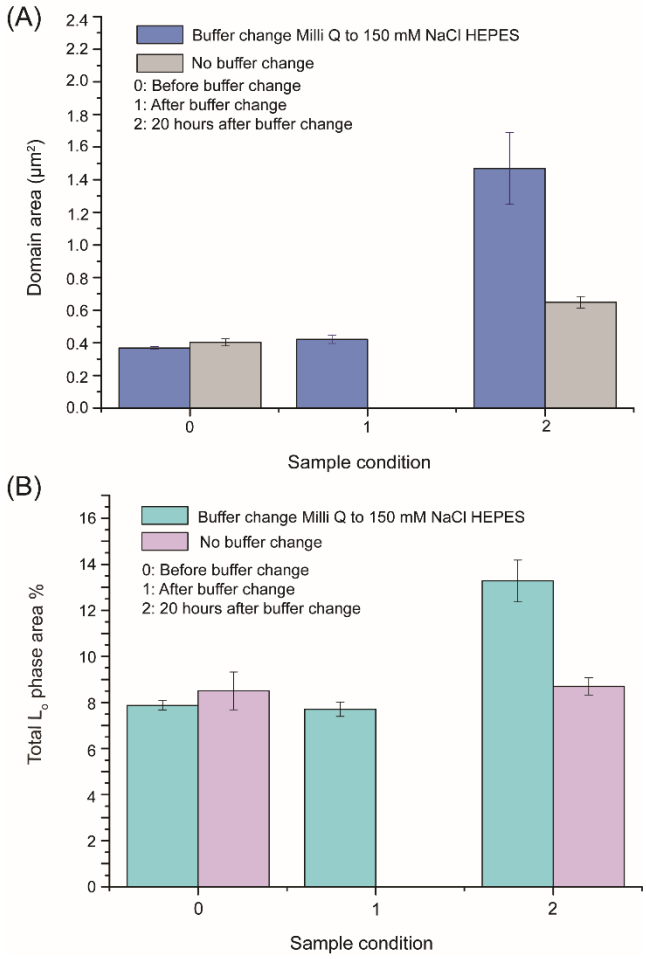

Figure S1. Average domain area (A) and total L_o phase area % i.e. percentage of area of the L_o phase with respect to the area of the entire image (B) before (0), immediately after (1) and 20 hours after (2) replacing Milli Q water with 10 mM HEPES 150 mM NaCl buffer (blue and cyan in panel A and B, respectively) in comparison to control experiments with no buffer change (grey and purple in panel A and B, respectively). Clearly, upon buffer exchange, the average domain area and total L_o phase area % increase, whereas SLBs left in Milli Q water did not show any significant change of the average domain area or the total L_o phase area %.

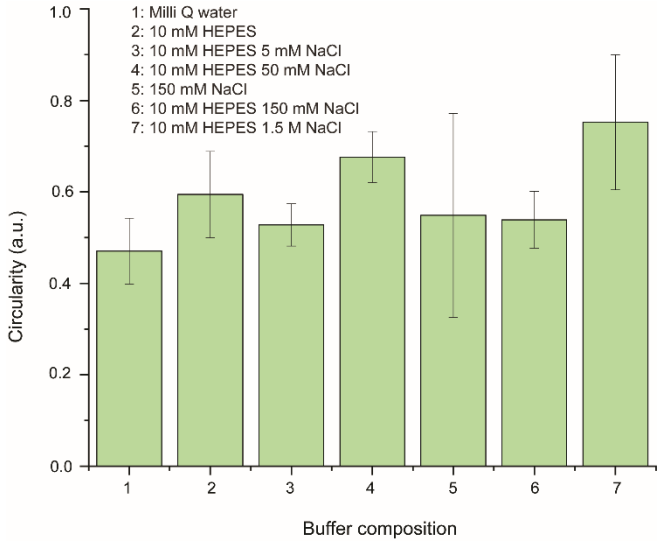

Figure S2. Circularity parameter, which is indicative of line tension along the domain boundary, as a function of SLB buffer composition. The domain circularity was determined using FIJI (ImageJ) software from minimum 10 confocal images from each of at least three SLBs prepared at each buffer composition[1]. The error bars stand for the standard deviations calculated

from the circularity values for all images at a particular buffer composition. No significant changes were observed when changing buffer composition, indicating that the PC-SM interactions do not get significantly affected by the addition of NaCl salt.

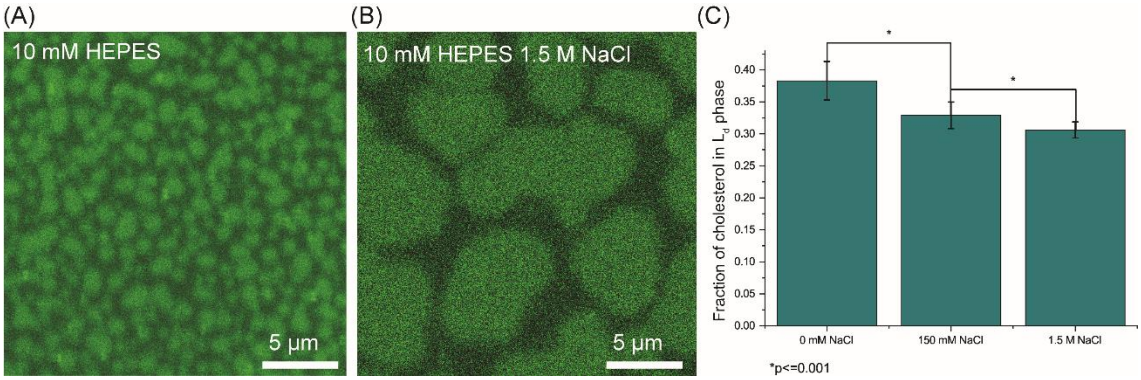

Figure S3. Phase separated SLBs with fluorescently labeled cholesterol analog (TopFluor cholesterol) were imaged in 10 mM HEPES buffer containing 0 mM, 150 mM and 1.5 M of NaCl. The fraction of cholesterol in the L_d phase was calculated as $I_{L_d}/(I_{L_d}+I_{L_o})$, where $I_{L_d}$and $I_{L_o}$are the intensity in L_d and L_o phase, respectively. Minimum 5 L_d and 5 L_o phase areas from at least 5 images for each buffer composition were analysed using FIJI (Imagej) software[3]. The error bars represent the standard deviation of the calculated fractions. Statistical analysis (t- test) confirms that the calculated L_d fractions for different buffer compositions are significantly different.

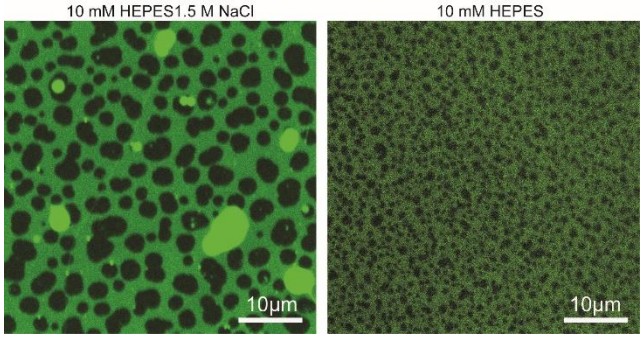

Figure S4. Confocal images of SLBs prepared in 10 mM HEPES buffer with 1.5 M NaCl (left) and without NaCl (right) after removal of bulk water and equilibration to 85% relative humidity (RH). The L_o domain size is bigger at higher NaCl concentration. No other structural changes were observed after removal of bulk water, except the variation in domain size, analogously to fully hydrated membranes in different buffer conditions.

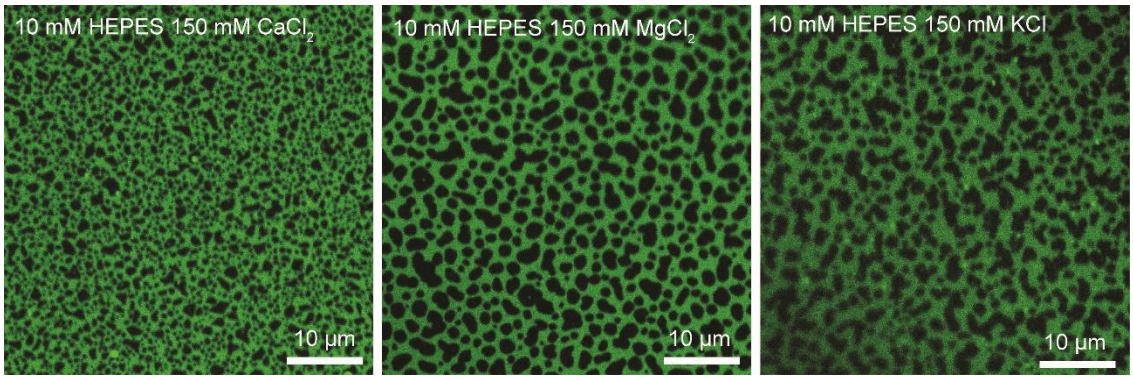

Figure S5. Representative fluorescence image of the fully hydrated, phase-separated SLBs prepared in the presence of 10 mM HEPES 150 mM $CaCl_2$, 10 mM HEPES 150 mM $MgCl_2$ and 10 mM HEPES 150 mM KCl buffer.

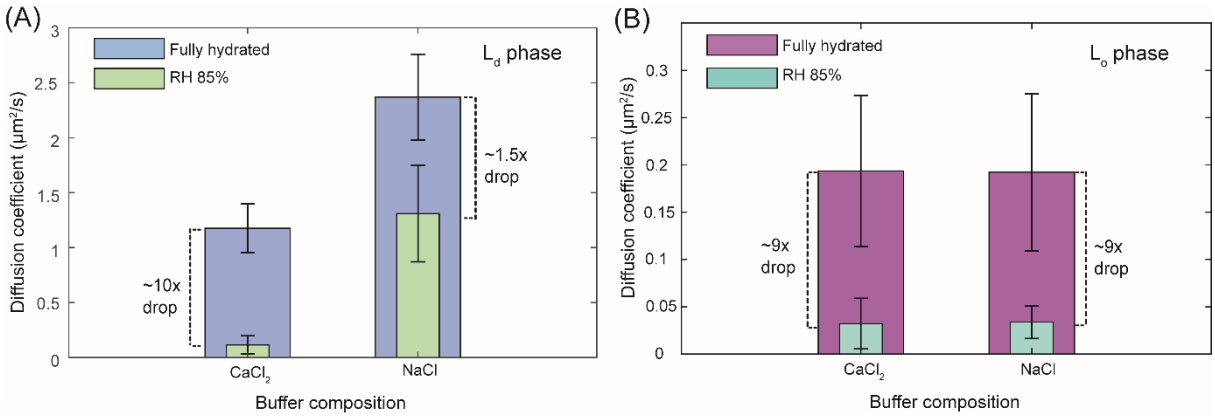

Figure S6. The average diffusion coefficient (*D*) values for 1:1 egg SM/cholesterol L_o phase SLBs in the presence of 10 mM HEPES buffer with addition of 150 mM NaCl or 150 mM $CaCl_2$ (B) in comparison to the L_d phase (A) at fully hydrated condition and at 85% RH.

References

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