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Supporting Material

Changes in single K^+ channel behavior induced by a lipid phase transition

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Supporting Methods

Supported Lipid Bilayer Preparation. Supported lipid bilayers were prepared by the vesicle fusion technique. The protein-lipid suspension was sonicated for 30s in an ultrasonic bath to obtain small unilamellar proteo-liposomes (SUVs). Then we added 70 µl of our proteoliposome suspension on a freshly cleaved piece of mica. The lipid suspension was incubated for 15 min at 32°C and then rinsed abundantly with 450 mM KCl, 25 mM Hepes, pH 7 buffer solution. The solution was then exchanged for the imaging solution (150mM KCl, 10mM potassium dihydrogen citrate at pH 7) by extensive rinsing. Then the mica support with the formed lipid bilayer was mounted on the temperature-controlled stage of the AFM.

Atomic Force Microscopy. Atomic Force Microscopy (AFM) experiments on supported lipid bilayers with reconstituted KcsA proteins were performed with a Bioscope equipped with a Nanoscope IIIA controller (Veeco Metrology, USA). Imaging was performed in tapping mode at a scan rate of 1-2 lines/s using triangular silicon nitride cantilevers (Olympus OMCL-TR400PB-1, Japan) with a nominal spring constant of 0.09 N/m and a resonance frequency in liquid between 8-9 kHz. The force applied to the membrane was adjusted to the lowest possible value allowing reproducible imaging. The sample temperature was continuously monitored by a digital thermometer Fluke 16 (Fluke, Italy) equipped with a small K-thermocouple probe (Thermocoax GmbH, Germany) in direct contact with the imaging buffer.

Supporting Results

Comparison between KcsA single channel conductance and DSC traces

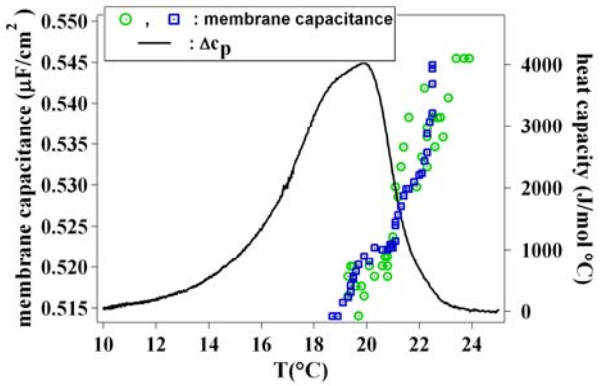

Fig. S1: Plot of the lipid bilayer capacitance as a function of temperature for a POPE:POPG 3:1 mixture in the Montal-Muller configuration (pH 3:pH 6.5). The green and blue points refer to two different measurements. The data have been obtained for a temperature dowscan. In the plot the downscan DSC trace for a POPE:POPG 3:1 lipid mixture at a pH 3 has been overlaid to the capacitance data. The DSC trace has been shifted by 1.5°C to lower temperature in order to align the rising of the excess heat capacity profile with the decrease of the capacitance.

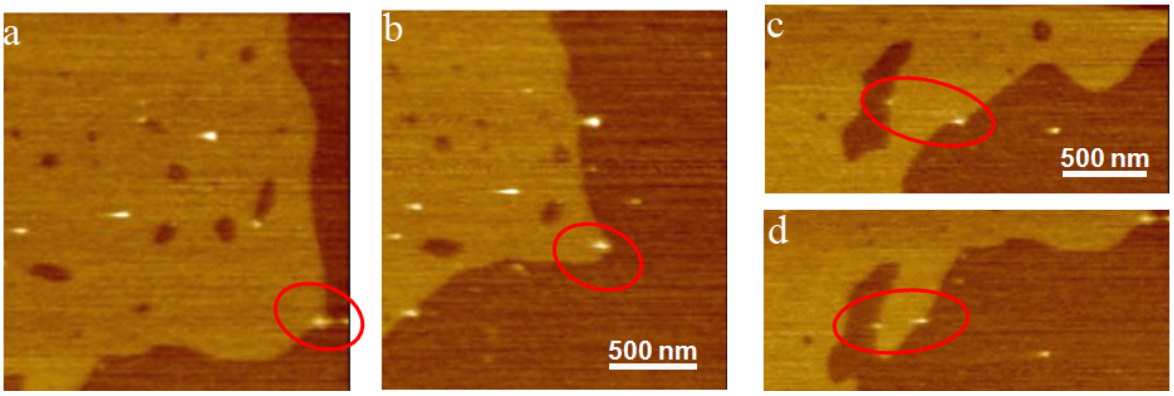

Fig. S2. Redistribution of KcsA upon domain melting. (a) At 18.5 °C a KcsA protein (in the red ellipse) is aligned at the domain boundaries between the solid ordered and liquid disordered phase. (b) Upon heating to 19.5 °C the solid ordered domain melts and hence occupies a smaller area fraction. The protein stays aligned between the solid ordered/ liquid disordered interface. (c-d). The redistribution behavior of two KcsA proteins were followed in another area of the SLB and at other temperatures of (c) 16.5 °C and (d) 18.0 °C.

In Figs. 2d, 3c, d and 4 of the manuscript we compared functional parameters with the excess heat capacity profiles for both the POPE:POPG 3:1 and POPE:POPG 1:1 lipid mixtures. In all cases the excess heat capacity profiles had to be corrected by -1.5 °C. This can be explained by the geometrical differences between the planar bilayers (functional studies) and the liposomes (DSC) and a possible influence of hexadecane residues in the planar lipid bilayer. It should be stressed that the shift was systematic for both mixtures -1.5°C. We have followed changes in the bilayer's unit area capacitance during temperature changes in order to estimate the BLMs transition behavior (1). The data are reported in Figure S1. We could conclude that both systems display transition regimes in a similar temperature regime, with a shift of -1.5 °C being feasible. It should be pointed out that in the proximity of the melting phase transition of lipid bilayers many physical parameters change. These changes involve an enhancement of macroscopic fluctuations in enthalpy, volume and area (2). The fluctuation-dissipation theorem relates these changes to the heat capacity profile, meaning that the higher the excess heat capacity at a certain temperature the stronger are the fluctuations. In addition fluctuations slow down in the phase transition region (3, 4). This means that all physical bilayer properties which are related to the system's fluctuations display the same kinetics and strength. One of these parameters is the bilayer's compressibility. The lipid bilayer becomes softer in the phase transition regime, reaching a maximum at the temperature corresponding to the highest excess heat capacity. It is very likely that a protein's conformational changes involving the

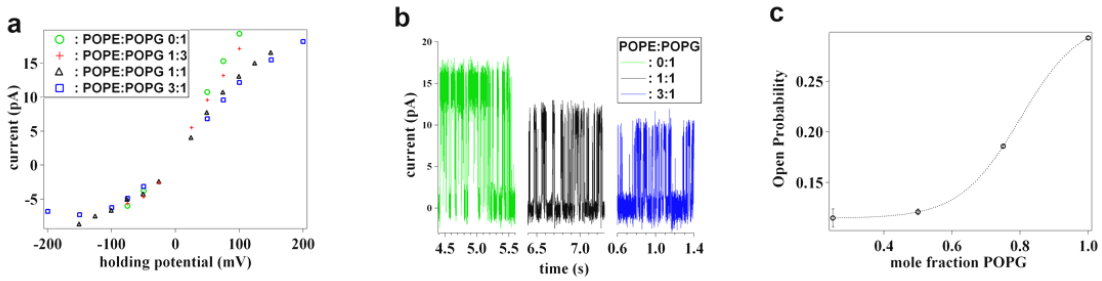

Fig. S3: Effect of different lipid composition on KcsA functionality. a) Effect of the lipid composition on the I-V curves. The experiments were performed under pH asymmetric conditions (pH 3, pH 6.5) and symmetric 150 mM K^+ concentration. b) Examples of traces of channel activity in the bilayers of different lipid composition. c) Effect of POPG mole fraction on the open probability of KcsA. Upon increasing the POPG fraction the KcsA open probability increases.

lipid/protein interface are fine-tuned by these physical properties.

Atomic Force Microscopy Experiments: Protein Redistribution in Dependence of Domain Coexistence

In a recent study we studied the redistribution behavior of KcsA protein reconstituted in supported lipid bilayers (SLBs) after the evolution of a solid ordered domain in a previously liquid disordered bilayer by decreasing the temperature (5). Following the evolution of the solid domains, the proteins which were previously uniformly distributed in the bilayer, segregated in the liquid disordered phase. In particular, the proteins had the tendency to align along the domain boundaries or eventually started to cluster in the liquid disordered environment. It should be noted that the observed behavior is in agreement with the hydrophobic matching principle. We have also performed heating experiments. In these experiments the SLB was rapidly cooled at the beginning of the experiment, a solid ordered domain was present and then we heated the sample again. Representative zooms of two regions of the same SLB are given in Fig. S2. In both regions it is possible to follow the melting of the solid ordered domain. At the same time the proteins aligned along the domain boundaries and in some cases they followed the retracting interface between lipid domains. This can be attributed to an attractive potential towards the domain interface acting on the protein (6).

Effect of POPG molar fraction on the KcsA single channel conductance

A variation of phospholipid molecules composing the bilayer which hosts the channels could result in a modification of the mechanical environment felt by the channels, so we investigated the KcsA unitary conductance variation as a function of lipid composition. To this aim we studied the single channel behavior of KcsA reconstituted in Black Lipid Membranes (BLMs) with different molar fractions of POPE:POPG: 3:1, 1:1, 1:3 and 0:1. Fig. S3 shows the results in terms of single channel conductance and open probability. All the measurements have been performed at 23°C and with an asymmetric pH configuration (pH 3 and pH 6.5). Increasing the fraction of POPG leads to higher single channel conductance and open probability. Similar results have been obtained by Marius et al. (7) using different molar ratios of POPC and POPG.

The results have been interpreted, as far as open probability is concerned, as due to the requirement for the nonannular sites of KcsA to be occupied by anionic lipids in order to open the channel. The presence of negatively charged lipids has in fact been reported to be essential for KcsA functionality (8) .

Effect of pH asymmetry on the behavior of KcsA upon temperature variation

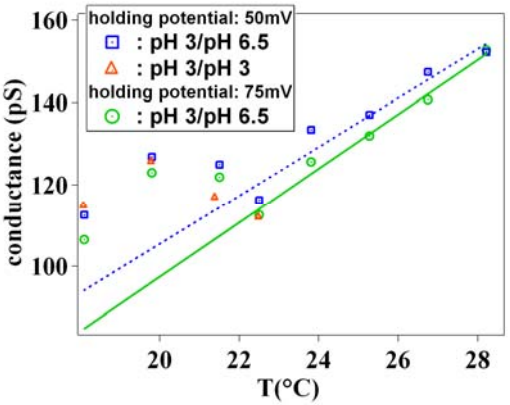

Fig. S4. Dependence of the KcsA single channel conductance upon temperature variation for different pH configurations. The first pH refers to the cis chamber and the second one to the trans chamber. The behavior is the same in both cases, meaning that pH 3 imposes its bilayer transition temperature. It is probable that an interleaflet coupling mechanism drives the transition at the same temperature when at least one of the two chambers is at pH 3

Comparison between open dwell-times in POPG and POPE:POPG 3:1

As reported by Marius et al. (7), for mixtures of POPC:POPG and by us for mixtures of POPE:POPG (see fig. S3) higher fractions of POPG result in an increase in ion channel conductance and open probability. Thus, in order to isolate the contribution of the lipid bilayer mechanical properties on the channel activity from contributions due for example to a variation in lipid surface charge density one

needs to apply other criteria. Hence, we measured also the kinetic parameters of KcsA. In Fig. S5a we show typical ion current traces taken at 21.5 °C when the KcsA protein was reconstituted in POPG only or in POPE:POPG 3:1 bilayers, whereas in Fig S5b we show the corresponding open dwell time histograms. Both the slow and fast open time contribution in the POPG bilayer increase from 10.4 ms and

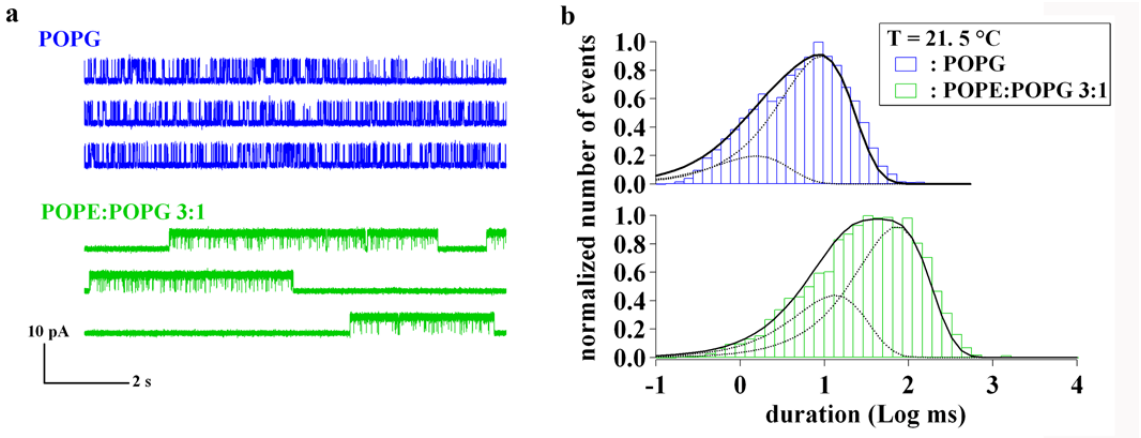

Fig. S5. Comparison between KcsA open dwell times in POPG and POPE:POPG 3:1 at 21.5 °C and at a holding potential of 50 mV. (a) Examples of ion current traces for KcsA in reconstituted in BLMs of POPG only and POPE:POPG 3:1. (b) Dwell-time histograms for the open states, for KcsA reconstituted into planar bilayers of POPG only and POPE:POPG 3:1. The open dwell-time distributions were fitted with exponential components (⋯) determining the overall fit (—).

1.8 ms to 84.5 ms and 15.3 ms. In the case of POPE:POPG 3:1 the maximum opening times which were determined at 20 °C increase further to values of 380 ms and 50 ms. Hence, the open times for channels in a pure POPG bilayer in the same temperature region are significantly lower than the open times in a POPE:POPG bilayer in the phase transition. We attribute this effect largely to the variation of the mechanical properties of the bilayer in the phase transition region.

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