Supplementary Information

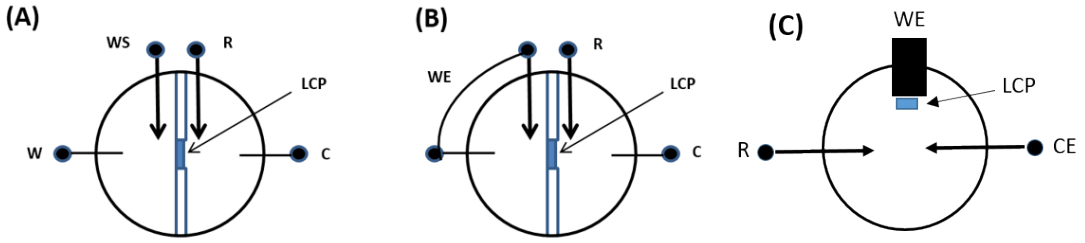
The EcCLC antiporter embedded in the lipidic liquid crystalline films – molecular dynamics simulations and electrochemistry

Przemysław Miszta[1,2#], Ewa Nazaruk[2#], Dorota Nieciecka[2], Mariusz Możajew[1,2,3], Paweł Krysiński[2*], Renata Bilewicz[1,2*], Sławomir Filipek[1,2*]

[1]Biological and Chemical Research Centre, University of Warsaw, Zwirki i Wigury 101, 02-089 Warsaw, Poland
[2]Faculty of Chemistry, University of Warsaw, Pasteura 1, 02-093 Warsaw, Poland
[3]Nencki Institute of Experimental Biology, Polish Academy of Sciences, 3 Pasteur Street, 02-093 Warsaw, Poland

Electrochemistry – instrumentation and procedures

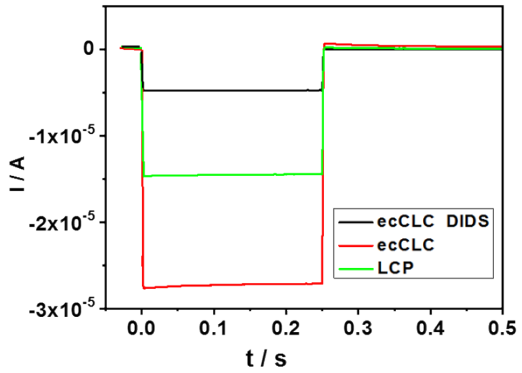
LCP membrane in a two-compartment Teflon cell separated with Teflon septum. Electrochemical impedance spectroscopy and chronoamperometry (CA) measurements with four electrode or three electrode arrangements, respectively, were carried out for a LCP with or without EcCLC protein placed in a 2 mm diameter orifice in a 3 mm thick septum, separating two chambers filled with aqueous solutions (Scheme S1). The LCP surfaces were made flat using a microscopic cover slide. For the case of the asymmetric configuration, the NaCl concentration or pH was varied across the LCP, as specified *infra*. For such a system, the transport of ions towards/from the orifice is fast as compared with the ions transport within the LCP separating two aqueous electrolytes and therefore the phenomena in the LCP are rate-limiting during the experiments. The 4-electrode cell setup (CH Instruments Model 600C Electrochemical Analyzer) is used for the precise control over potential across a membrane or junction potentials between two immiscible phases (Scheme S1A). In this configuration, the potential difference occurring as a result of a passage of charge (ions) across the LCP-filled orifice is controlled (measured between R and WS electrodes), giving the possibility to calculate the resistance or conductivity of the LCP. In 4-electrode mode, the potential biases due to any electrochemical reactions that may occur at the working and counter electrodes are eliminated. Instead, the response of the LCP separating two electrolyte solutions to the voltage bias (or the LCP structure/content) is being measured.



Scheme S1 Cell and electrode configuration for (**A**) electrochemical impedance spectroscopy (EIS) measurements, (**B**) chronoamperometry (CA) measurements using free-standing LCP, and **(C)** and the GC electrode covered with the LCP film.

In a 3-electrode mode, that was used for CA experiment for the same system of LCP separating two aqueous solutions, the Working Sense (WS) is coupled with Working Electrode (W), typically then denoted as Working Electrode (WE) (Scheme S1B). The Reference Electrode (RE) measures a potential drop across the LCP and solution between the LCP and WE. In this system two low resistance Ag,AgCl|3M KCl_{aq} were used as WE and R electrodes and Pt mesh as counter (C) electrode. The potential drop between WE and R electrodes was essentially localized within the LCP.

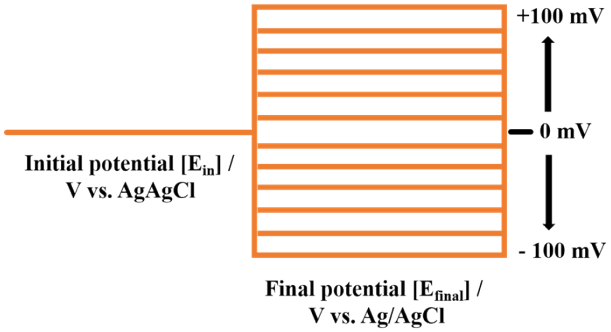
Due to the dynamic nature of LCP, the experimental protocol was always as follows: first, after an introduction of the bicontinuous LCP, and filling both compartments with aqueous electrolyte buffered with McIlvaine buffer, the system was left to equilibrate for 2 hours. This time lag was found necessary for stable and reproducible results, as it allows ions from aqueous buffer to equilibrate with free-standing LCP. Then the low-bias electrochemical impedance measurements were carried out within the frequency window of 10^4 to 0.1 Hz (0.005 V amplitude and potential range -0.12V to +0.12 V). To complete these experiments 5-6 hours were required. Next, the system was switched to a 3-electrode mode and CA data were acquired. Scheme S2 shows the potential steps applied in the CA experiment.



Scheme S2 Current responses of a free-standing LCPs. Potential step from 0 V to -120 mV, step duration 0.25 s. Labels: LCP – cubic phase alone; EcCLC – cubic phase with antiporter protein; EcCLC DIDS – inhibitor added on both sides of LCP. 150 mM NaCl supporting electrolyte, pH = 5.0 on both sides. See text for more details.

EcCLC in LCP film on glassy carbon electrode. Efficiency of chloride transport in LCP film was studied using chronoamperometry (CA). CHI bipotentiostat with a standard three-electrode

arrangement in buffered solution was used. Ag/AgCl was used as the reference electrode and a platinum foil as the counter electrode. The working electrode was glassy carbon electrode (GCE) modified with the LCP film (the surface area of GCE was 0.07 cm^2). The entire setup was placed in a Faraday cage to minimize external disturbances. Before the experiments, the working electrode was polished on alumina of decreasing size (from 0.3 µm to 0.05 µm) on a polishing cloth. The electrodes were subsequently sonicated to remove adhered alumina particles, rinsed with ethanol and water and left to dry. The thickness of the LCP layer was controlled by the design of working electrode and was kept constant (0.5 mm), so that the geometric volume of this layer remained the same during the experiments. CA on GCE covered with EcCLC was done in McIlvaine buffer pH 5 or 7.4 containing 150 mM NaCl and 5 mM DDM. The modified electrodes were immersed in a buffered solution and left for at least 1 h to stabilize. ecCLC ion transport properties were studied by chronoamperometry (CA) on GCE covered with LCP film with incorporated protein. Dependence of transient current on the applied potential steps (I-V curve) was measured, potential steps were applied from the initial potential (0 mV), to the final potential varied from -100 to +100 mV (Scheme S3).



Scheme S3. The potential steps applied in the CA experiment with GCE covered with LCP film. Potential step from 0 V to -100 mV, step duration 1s.

Impedance studies of free-standing LCP membrane placed in a Teflon septum. 4-e system

Free-standing films of LCP without or with EcCLC were investigated with use of electrochemical impedance spectroscopy (EIS), in hope to get a deeper insight into the ion transport mechanism at low potential bias up to +/- 140 mV. Similar EIS studies were proposed by Brown *et al.*[1] for pure phytantriol Q^{224} cubic free-standing films deposited in a micrometer hole in a micrometer thick septum separating two aqueous solution. However, apart from the thickness and diameter of our free standing LCP that are composed of MO lipidic molecules, our system differs from the above-mentioned in that it contains also the EcCLC $2Cl^-/1H^+$ antiporter protein. From the point of view of impedance experiments, the LCP with or without protein, can be considered as a porous dielectric barrier for ion movements through the interleaved aqueous channels. As such, its impedance could be modeled with an equivalent electrical circuit representing semi-infinite uniform transmission line.[2] Assuming the identical pore properties, this semi-infinite line can be reduced to a circuit representing a mesh containing the impedance of all pores (Z_{pores}) in parallel with the external flat surfaces (C_s), all in series with the electrolyte resistance R_s. Impedance measurements were performed in 4-electrode

configuration, typical for membranous or liquid junction studies. They were carried out in +/– 140 mV potential range with 10 mV potential step (0.1 Hz to 10 kHz, 5 mV amplitude).

What can be immediately learned from the Bode plots of our results is that the LCP separating two aqueous solutions, regardless of solution pH or the presence or absence of EcCLC behaves predominantly as ionic resistor with negligible capacitive contribution at high frequency range. This is exemplified with Bode plots of |Z| and phase shift versus logarithm of frequency (Fig. S1).

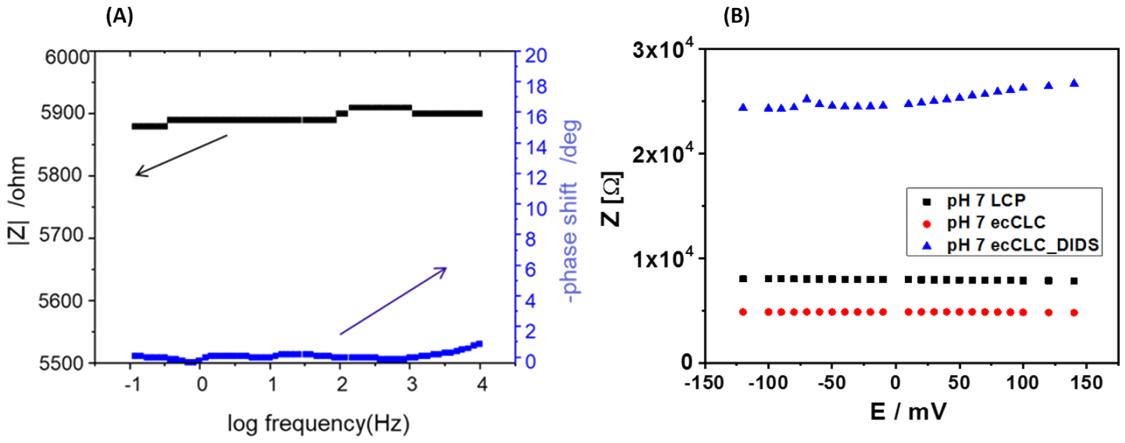


Fig. S1 (**A**) Example of Bode plots for free-standing LCP membrane with EcCLC at pH 5 and 50 mV potential across the cubic phase. Supporting electrolyte: 0.150 M NaCl aqueous. (**B**) representative plots at pH 7 of |Z| vs. applied potential for pure LCP (black filled squares), LCP with EcCLC (red filled circles) and the same LCP with EcCLC after DIDS was introduced on both sides (blue filled triangles). All data were extracted from the Bode plots at 10 Hz.

For the case of capacitive contribution to the observed processes the phase shift close to 90° should be observed, at least within a certain frequency range. The values of |Z| correspond directly to the ionic resistance and can be compared with the values obtained from CA, whereas, as seen in Fig. S1A, the phase shift close to zero value over the whole frequency range show no capacitive contribution. We have used the real part of impedance data to plot them as a function of potential applied across the cubic phase placed in the septum. These plots, measured at 10 Hz, are shown in Fig. S1B for pH 7, in the absence of EcCLC protein, in the EcCLC protein incorporated in the LCP walls and, finally, with protein blocker, DIDS hydrolysis product – DADS. For pH 7 these results correspond well with the data obtained with CA technique (Figs. 11 and 12, main text), showing that the presence EcCLC decreases the resistance of LCP (CA current increases) and the addition of DIDS to both sides of LCP results in the resistance increase, even beyond the value for pure LCP. These results confirm the EcCLC protein channel action increasing of the ionic conductance of LCP, substantiating the possible use of LCP film for sensing and drug screening applications.

Effect of pH on Cl^- transport in electrode supported LCP films

From the current vs. potential plot the difference in current passing through EcCLC LCP was evaluated and the pH dependence of Cl^- transport was demonstrated (Fig. S2A). At pH 5

transport of chlorides is more effective than at pH 7, this behavior confirms that EcCLC is an antiporter, exchanging protons and chloride ions. The EcCLC membrane proteins transports chlorides more efficiently at more acidic pH, the resistance of the film (measured from the slope of I-V curve), decreases at pH 5. In the presence of EcCLC in LCP and 150 mM chloride ions at pH 5 the resistance is equal to ca 400 kΩ and increases significantly in pH 7 (1 687 kΩ). The proton flux seems to control the transport of chlorides – the current is always larger when negative potentials are applied to the electrode. This difference is clearly seen in Fig. S2B at potential close to 0 V e.g. current at -25mV is 100 nA while that at +25 mV is only 20 nA. Thus, H^+ permeation pathway is stimulated by appropriate potential.

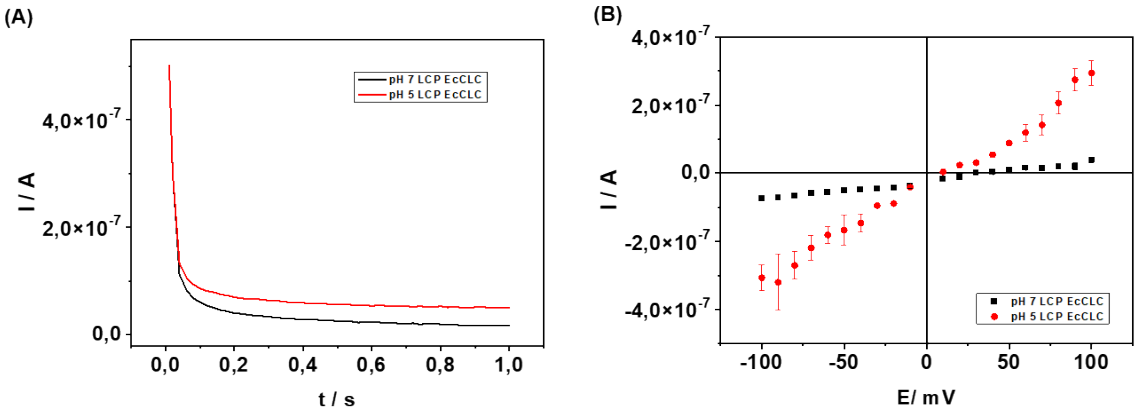


Fig. S2 Chronoamperometry at GC electrode covered with the EcCLC-containing LCP film. (**A**) Representative CA curves for GC covered with EcCLC_LCP at pH 5 and 7.4. Final potential 40 mV. (**B**) Plots of CA current measured 1 s after different potentials application.

References

1. R. Brown, E. Madrid, R. Castaing, J. M. Stone, A. M. Squires, K. J. Edler, K. Takashina and F. Marken, *ChemElectroChem*, 2017, **4**, 1172-1180.
2. A. Lasia, in *Electrochemical Impedance Spectroscopy and its Applications*, Springer-Verlag New York, 2014, DOI: 10.1007/978-1-4614-8933-7, pp. 203-250.