

# Electrochemical noise analysis to probe ion transport mechanisms in a membrane channel

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## I. INTRODUCTION

Electrochemical noise (EN) analysis, defined as the evaluation of the spontaneous fluctuations of current generated by electrochemical processes<sup>1</sup>, has been used over the years to study kinetic effects in biological systems like neuronal networks<sup>2</sup> or biomembranes<sup>3</sup>. In particular, EN has been successfully employed in protein ion channels to investigate the transport mechanisms that control the channel function<sup>2,4,5</sup>. In this work we use EN to assess the different transport mechanisms occurring in the bacterial porin OmpF, a wide and weakly selective channel in the outer membrane of *E. coli*.

Noise analysis has been previously used in OmpF to investigate the pH titration of the channel residues<sup>4</sup>. It was shown that the power spectral density could be represented by a sum of Lorentzians plus the background spectrum, demonstrating that the conductance dynamics follow a two-state Markov process. Although the use of simple Markov models provides in principle all the kinetic constants and the number of independent residues participating in the process<sup>6,7</sup>, the study in this case was seriously complicated by the pH dependence of the current steps between substates, which should be constant to allow this kind of analysis.

Here we follow a complementary approach based on the noise studies of Hoogerheide et al. in synthetic nanopores<sup>8</sup>. We pay attention to the appearance of an additional white noise in the low frequency range, as shown in Fig. (1). The level of this constant region changes with the applied voltage bias so that higher potentials yield higher values of the white noise. The current fluctuations  $S_I$  obtained from the framed region of the PSD in Fig. (1) (right) can be related to the conductance fluctuations,  $S_G$ , by  $S_I = (S_G/G^2) \cdot I^2$ . The parabolic coefficient  $S_G/G^2$  contains relevant physicochemical information of the intrinsic system fluctuations. In particular, it has been related to the number of particles that fluctuates at the pore walls<sup>8</sup>.

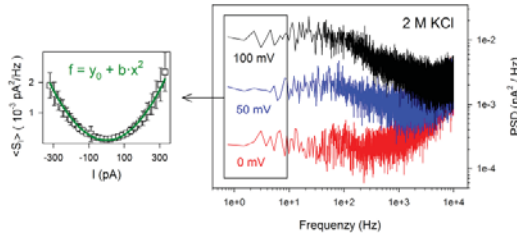


FIG. 1. Right: Power spectral density (PSD) calculated from single-channel current recordings at 2 M KCl electrolyte solution and different voltages. Left: parabolic dependence of the averaged PSD with current (open squares) obtained from the low frequency region (0 - 20 Hz band) framed in the right. The parabolic coefficient  $S_G/G^2$  is obtained from a parabolic fit (solid line).

Consequently, we performed a parabolic fit of the averaged low frequency noise  $S_I$  plotted versus the current  $I$  (Fig. (1), left) to obtain the parabolic coefficient  $S_G/G^2$  from each EN analysis. We performed this analysis with different electrolytes and studied how these fluctuations, altogether with the measured conductance, depend on ionic concentration to disclose the different transport mechanisms occurring in the OmpF channel.

## II. MATERIAL AND METHODS

We analyze the current fluctuations from single-channel measurements performed using the V-clamp technique. The detailed experimental protocol can be found elsewhere<sup>4</sup>. The lipid membrane was created with 1,2-diphytanoyl-sn-glycero-3-phosphocholine (DPhPC) lipid. The electrolyte (monovalent or divalent) solution was kept at pH 6. The bacterial porin OmpF was kindly provided by Dr. S. M. Bezrukov (NIH, Bethesda, USA). Single-channel current recordings were obtained using an Axopatch 200B amplifier (Molecular Devices, Sunnyvale, CA) with an in-line low-pass Bessel filter at 10 kHz. Data were digitalized with a sampling frequency of 50 kHz using a Digidata 1440 (Molecular Devices, Sunnyvale, CA). The chamber was isolated from external noise sources with a double metal screen (Amuneal Manufacturing Corp., Philadelphia, PA).

## III. RESULTS AND DISCUSSION

Fig. (2) displays the results obtained for both the (normalized) conductance (a) and the parabolic coefficient (b) as a function of ion activity, for the different electrolytes used. The use of electrolyte activity instead of concentration is necessary to separate the role of the channel from the intrinsic properties of the electrolyte that become important for salts of divalent cations at high molarity<sup>9</sup>. Fig. (2a) demonstrates that the channel conductance measured in salts of monovalent and divalent cations displays common trends. This is consistent with the lack of specificity of the OmpF channel reported in previous studies<sup>4</sup>. In all electrolytes under study the conductance scales as  $G \sim a^{3/4}$  for low activities. In the high activity regime we find saturation or even a slightly decrease in conductance that scales as  $G \sim a^{-1/4}$ . In the case of KCl this late regime is not attained because of the solubility limit. To rationalize these results, we used a simple theoretical model that accounts for: *i*) The effect of the charged residues of the pore walls -that induces the accumulation of counterions and the exclusion of coions to preserve local electroneutrality-, through the Donnan formalism. *ii*) The experimental dependence of diffusion coefficients on ion activity, which shows different variants distinctive of each electrolyte<sup>9</sup>. *iii*) The variation in the concentration of mobile ions inside the channel due to adsorption processes, using the Langmuir adsorption isotherm<sup>10</sup>.

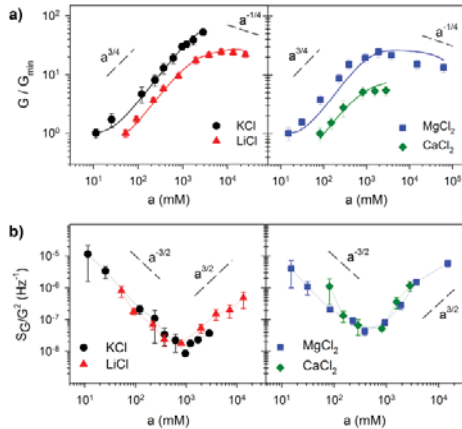


FIG. 2 a) Normalized OmpF channel conductance versus electrolyte activity for different salts. The dashed lines show the qualitative trends observed. The solid lines are calculated applying a theoretical model as described in the main text. b) Dependence of the parabolic coefficient  $S_G/G^2$  on electrolyte activity, for different salts. The dashed lines show the two regimes observed. The solid lines are drawn to guide the eye.

The existence of adsorption processes in OmpF has been suggested by a number of different approaches that pointed to the existence of a binding site for cations located around the central constriction of the OmpF channel<sup>11</sup>. In spite of all this information, the functional role of that binding site remained unclear since no blocking events have been observed and the channel displays Ohmic conduction in all conditions studied.

The theoretical model developed has only two free parameters: the channel fixed charge concentration,  $X$ , and the binding constant  $K_d$  of the binding model. Despite the simplicity of this approach, the model reproduces the two trends observed in the experiments, as reported in Fig. (2a) (solid lines). The parameters used for the calculation,  $X \sim 50\text{--}100$  mM and  $K_d \sim 70$  M, are of the order of magnitude expected. In any case, the qualitative message is clear: the fixed charge of the pore exerts a control of the ion transport and this control is enough to explain the behavior of conductance at the low activity regime, regardless the type of ions present in the system. In addition, the existence of a binding site for cations, altogether with the solution effects, explains the trend at high activities.

The information obtained from the parabolic coefficient, shown in Fig. (2b), can be understood using the same theoretical approach. For all electrolytes under study, the parabolic coefficient exhibits two different regimes,  $S_G/G^2 \sim a^{-3/2}$  at low activities and  $S_G/G^2 \sim a^{3/2}$  at high activities. Interestingly, the inflection point is observed around  $a \sim 1$  M, similarly to the onset of conductance saturation in Fig. (2a). From  $S_G/G^2$  we calculated the number of particles that contribute to the conductance noise,  $S_G$ , yielding  $S_G \sim a^0$  at the low activity regime. This means that the number of fluctuating particles remains constant when increasing the activity. Having in mind that only for surface-bound fluctuations does the parabolic coefficient  $S_G/G^2$  scale as  $a^{-3/2}$ <sup>8</sup>, the fluctuating particles could be just the counterions screening the channel charges located in the pore surface as explained by Donnan equilibrium. This result is consistent with a transport regime in which the electroneutrality requirements arising from the channel fixed charge dictate the channel conductance. In the

case of high activities,  $S_G \sim a^1$ . This result can be understood invoking again the binding processes that dominate the channel conductance in the high activity regime as shown in the analysis of Fig. (2a). We can assume that the fraction of occupied sites is directly related to the quantity of fluctuating particles that contributes to the noise. Using the Langmuir adsorption isotherm<sup>10</sup> to account for that binding, the fraction of occupied sites varies linearly with solution activity when the activity of cations is lower than the effective dissociation constant  $K_d$ . Accordingly, the conductance fluctuations are expected to follow  $S_G \sim a^1$ , which is what we actually observe in Fig. (2b). This explanation is consistent with the high  $K_d$  values obtained.

Therefore, by means of a particular EN analysis technique combined with conductance experiments we have demonstrated the existence of two main ion transport regimes in the OmpF channel, both for salts of monovalent and divalent cations.

## IV. CONCLUSIONS

The analysis of EN provides direct functional evidence of the transport mechanisms occurring in the protein channel OmpF showing features that may appear undetected in the current traces. This is especially evident at high salt concentration. In contrast to conductance experiments, EN analysis show a well-defined common trend clearly visible in all electrolytes under study. The adsorption processes related to the binding site for cations that are hardly visible in conductance experiments can be detected and elucidated using EN analysis.

## ACKNOWLEDGEMENTS

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