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

Maria Queralt-Martin, *M. Lidón López and Antonio Alcaraz*

Laboratory of Molecular Biophysics, Department of Physics
Universitat Jaume I, Castellón, Spain

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Outline

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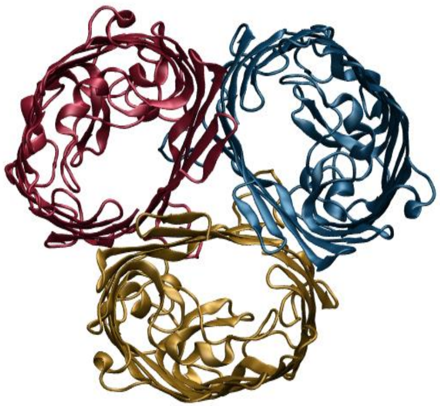
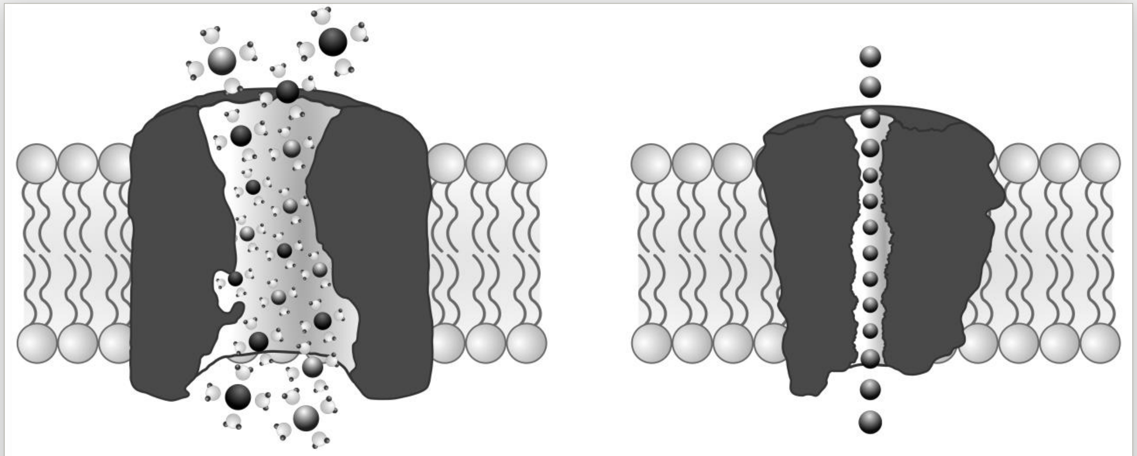
1. Introduction
2. Noise analysis in the bacterial channel OmpF
3. Results obtained
4. Conclusions and open questions

The OmpF channel

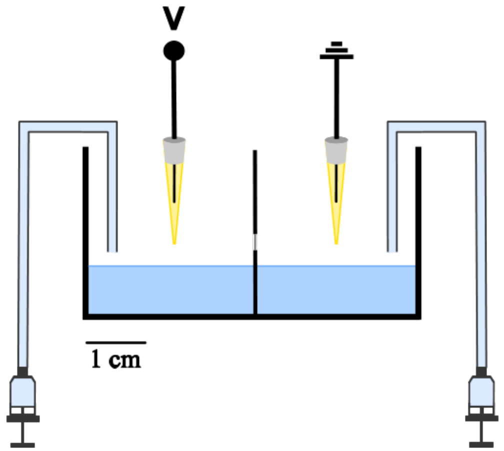
- **Protein that forms trimeric channels**
- **Located at the outer membrane of *E. coli***
- **Wide pores with multiionic transport**

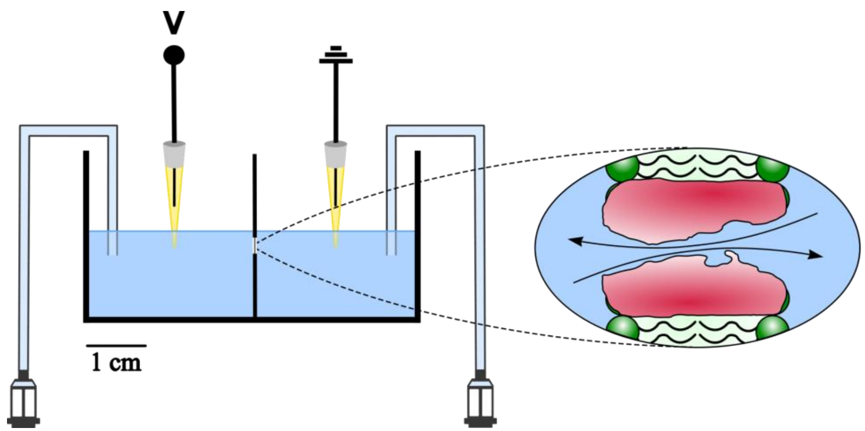




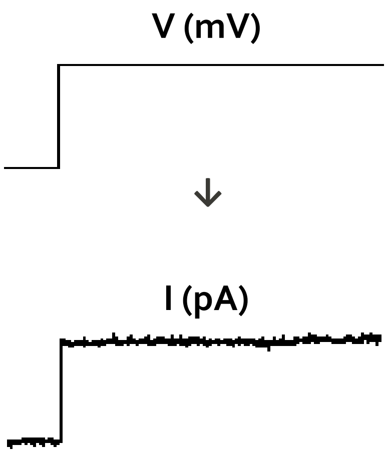
Experimental technique

Planar Bilayer Electrophysiology
Bilayer formed by apposition of monolayers



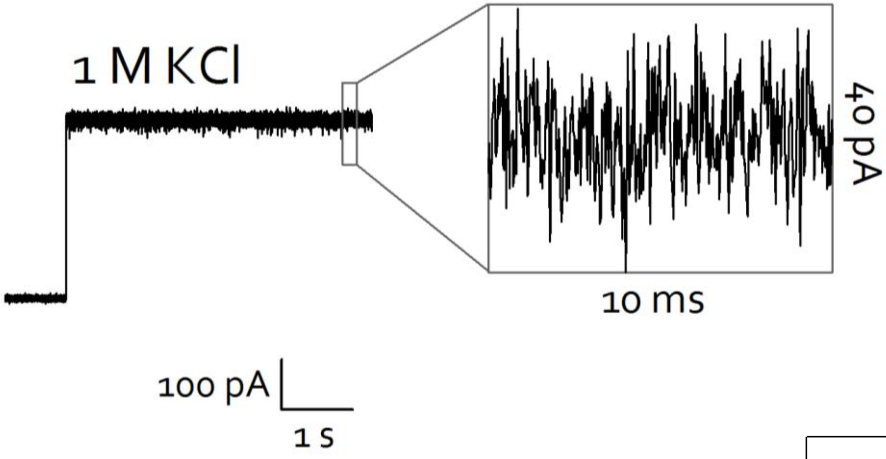


Measurement of single-channel current

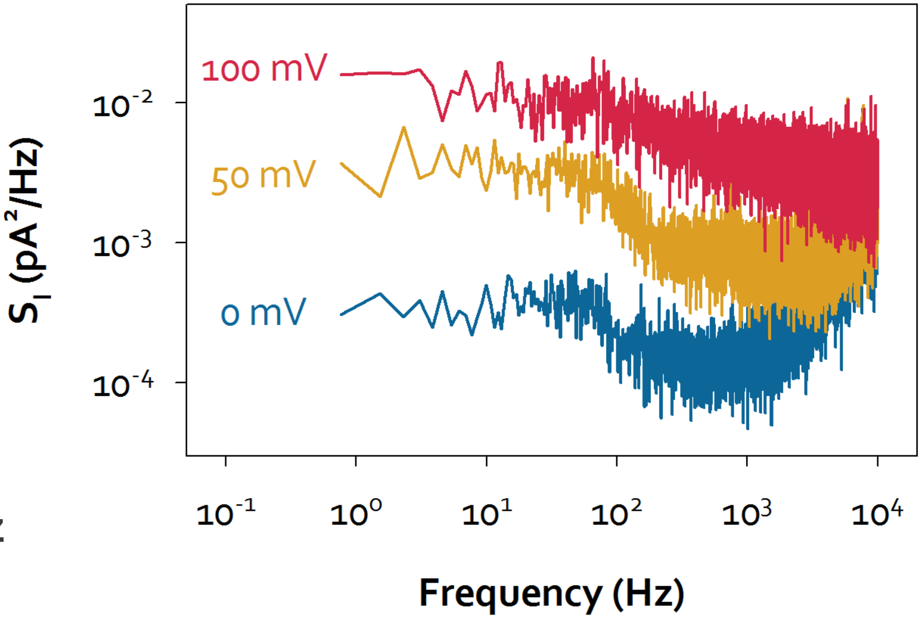


Detailed study of the transport properties of protein channels

Noise analysis in OmpF channel



Study of current fluctuations



Sampling rate: 50 kHz
Low-pass Bessel filter at 10 kHz
Inset: 500 Hz Bessel filter

Noise analysis in OmpF channel

Previous studies of current fluctuations in OmpF

Lorentzian curve

$$S(f) = \frac{4(\Delta i)^2 \tau^2}{\tau_1 + \tau_2} \frac{1}{1 + (2\pi f \tau)^2}$$

$$\tau = \frac{\tau_1 \tau_2}{\tau_1 + \tau_2} \qquad \tau_1 = 1/k_{\text{off}}$$

$$\tau_2 = (k_{\text{on}}[H^+])^{-1} = 10^{\text{pH}}/k_{\text{on}}$$

⬍

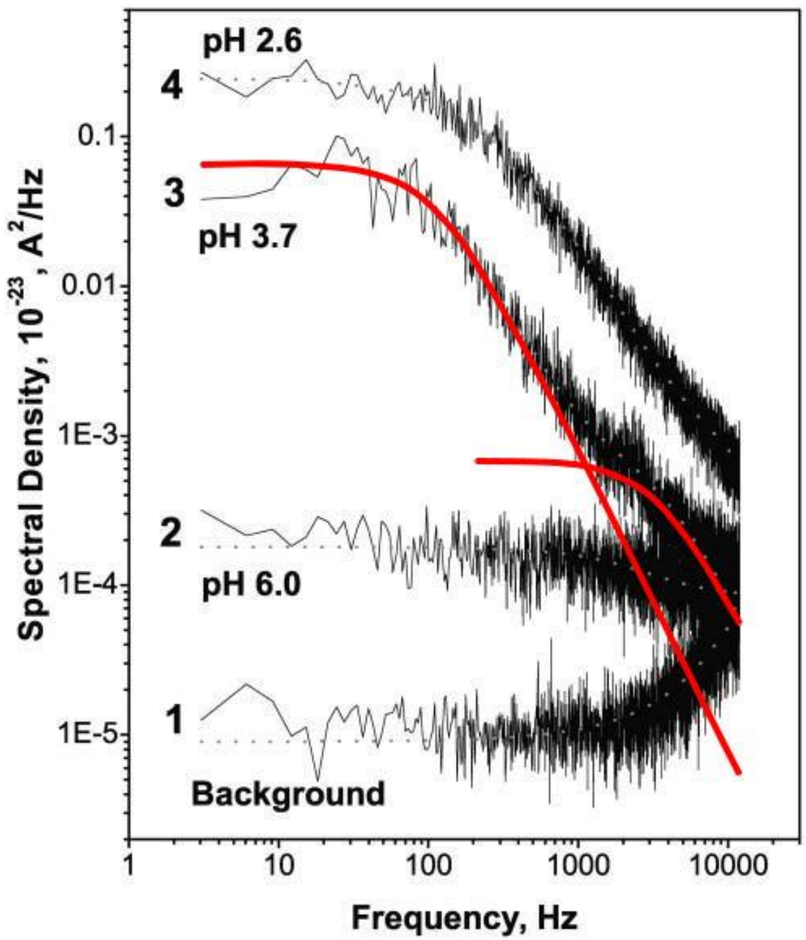
Two-state Markov process

Simple model for first-order protonation reactions

$$A + \mathrm{H}^+ \underset{k_{\text{off}}}{\overset{k_{\text{on}}}{\rightleftharpoons}} B$$

Problem

Current steps between substates (Δi) are not constant



Nestorovich et al. *Biophys. J.* 2003
Bezrukov et al. *Phys. Rev. Lett.* 1993

Noise analysis in OmpF channel

Alternative approach for the study of current fluctuations in OmpF

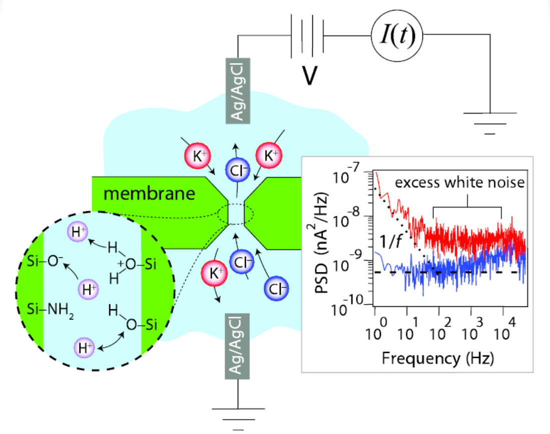
PRL **102,** 256804 (2009) PHYSICAL REVIEW LETTERS week ending 26 JUNE 2009

Probing Surface Charge Fluctuations with Solid-State Nanopores

David P. Hoogerheide, Slaven Garaj, and Jene A. Golovchenko
Physics Department, Harvard University, Cambridge Massachusetts 02138, USA
(Received 8 April 2009; published 26 June 2009)

Conductance fluctuations S_G:

The PSDs of the voltage-dependent noise sources scale as V^2



For an Ohmic system (G=I/V)

$$S_I = S_G \cdot V^2 = \left(\frac{S_G}{G^2}\right) \cdot I^2$$

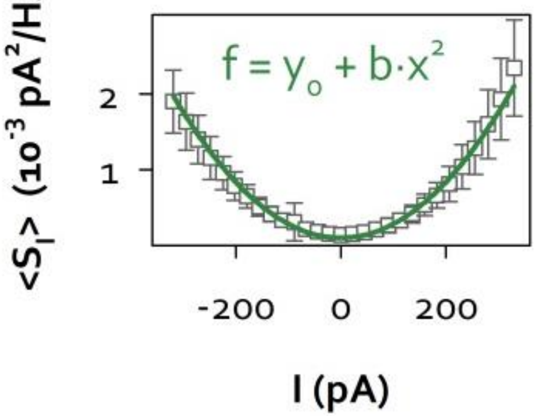
Parabolic coefficient

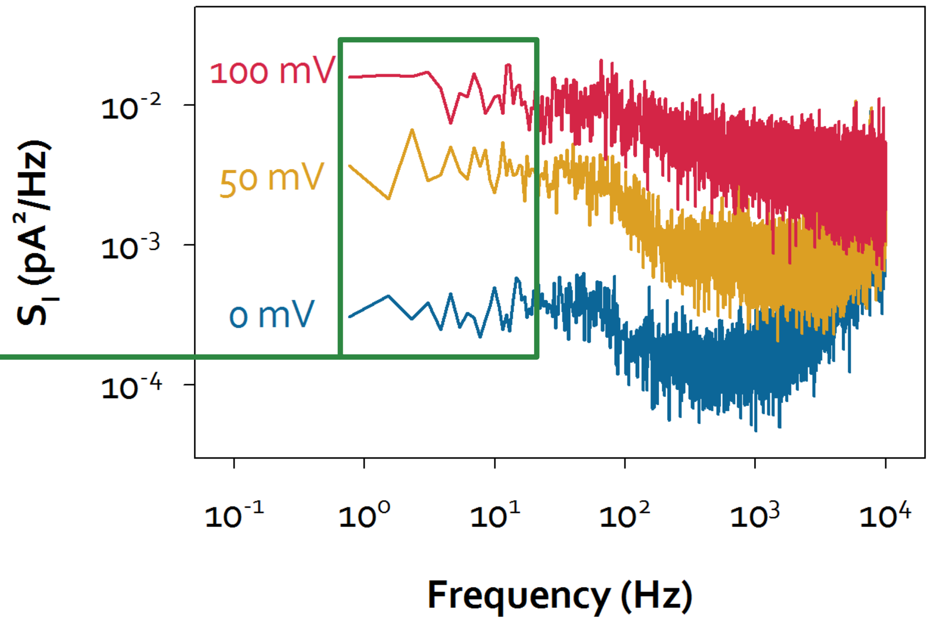
The electrical current through the channel reveals fluctuations in conductance

Noise analysis in OmpF channel

Alternative approach for the study of current fluctuations in OmpF

Parabolic coefficient S_G/G^2





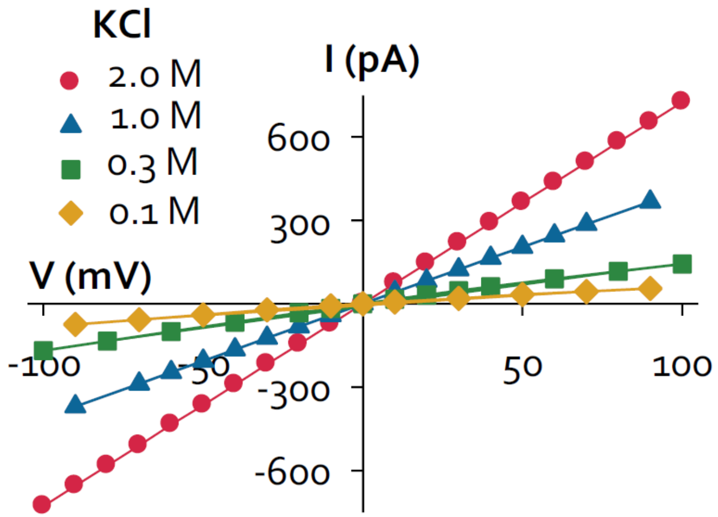
Conductance fluctuations S_G:

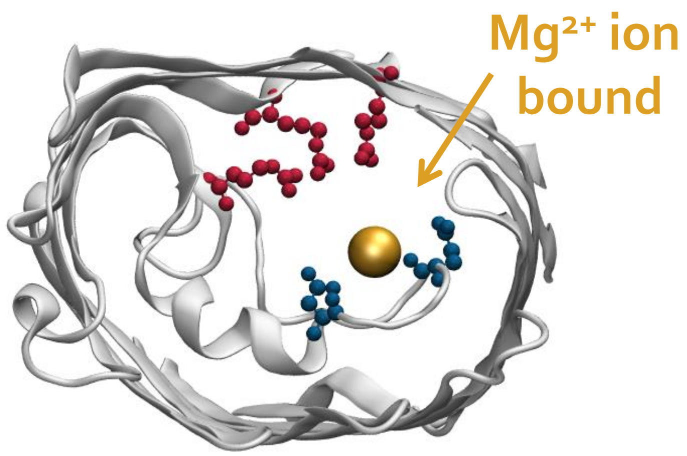
$$S_G = \left(\frac{S_G}{G^2}\right) \cdot G^2$$

We evaluate conductance fluctuations without knowing the exact mechanism behind them

Exploring different conditions

Different electrolytes: **KCl, LiCl, MgCl₂, CaCl₂**
Different concentrations: **10 mM – 6 M**





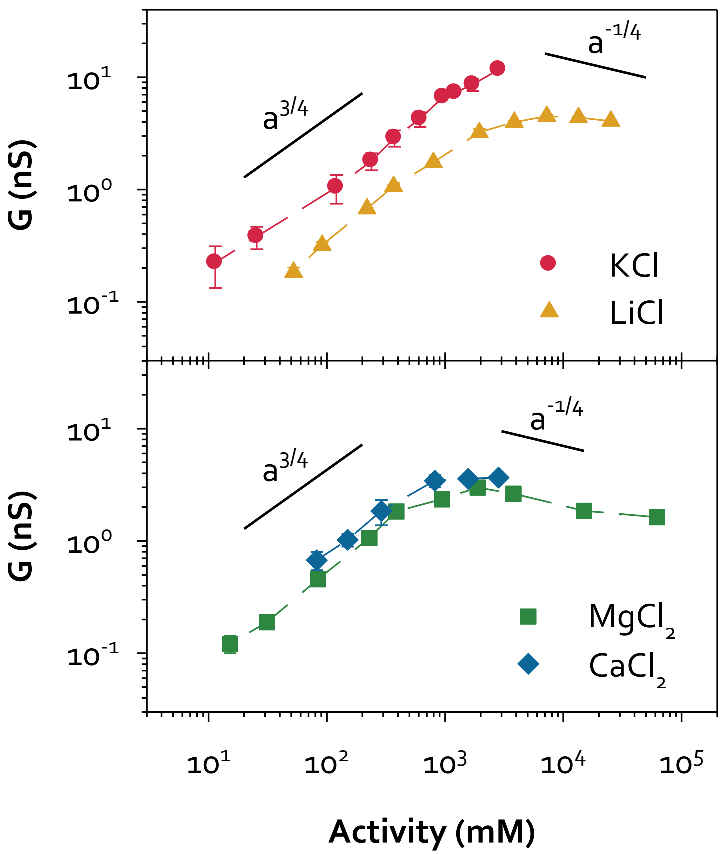
1 M MgCl₂
Yamashita et al. *EMBO J.* 2008

Noise analysis as a complement of previous studies with conductance and selectivity

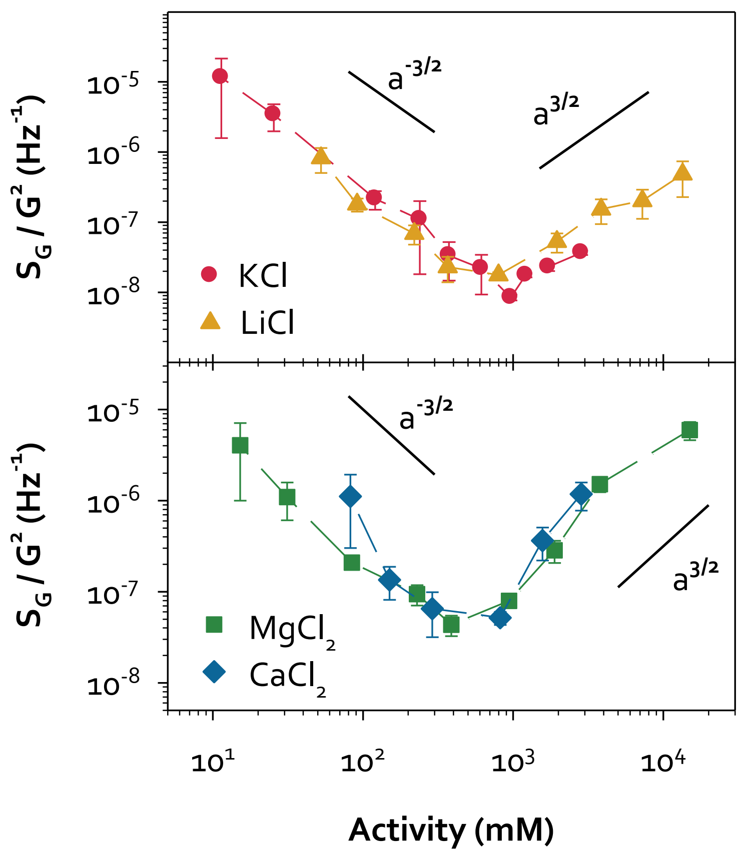
Exploring different conditions

Different electrolytes: **KCl, LiCl, $MgCl_2$, $CaCl_2$**
Different concentrations: **10 mM – 6 M**

Conductance

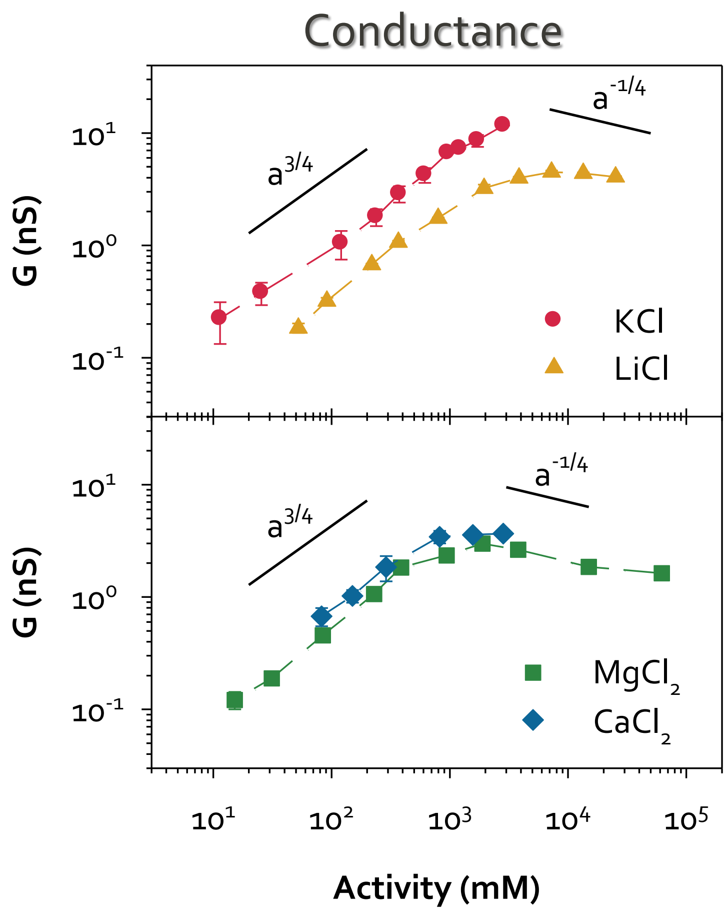


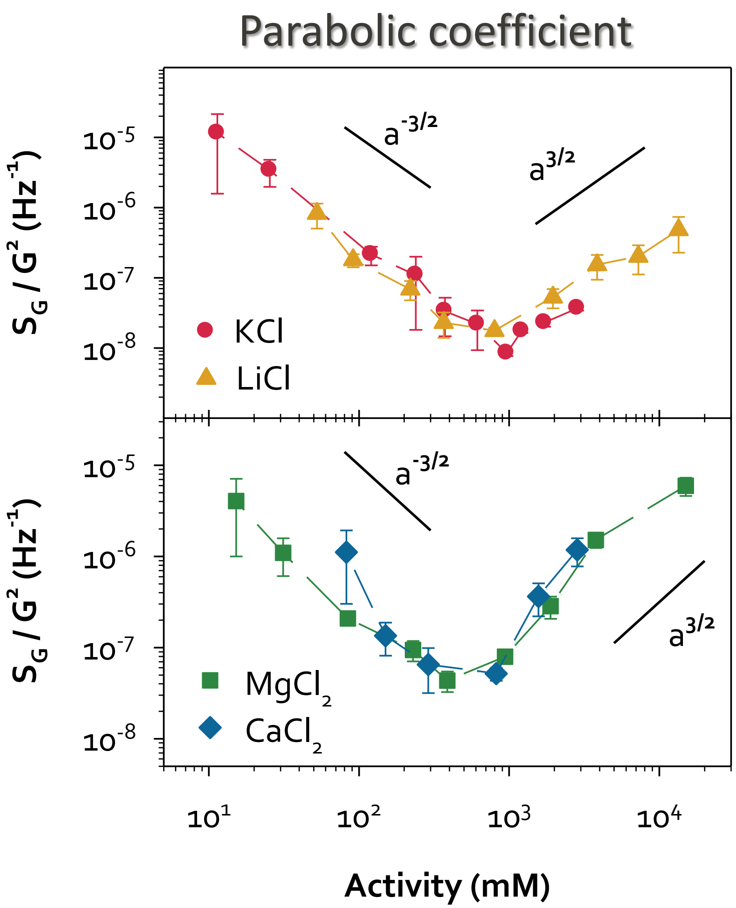
Parabolic coefficient



Exploring different conditions

Similar pattern for all salts under study & Two characteristic transport regimes

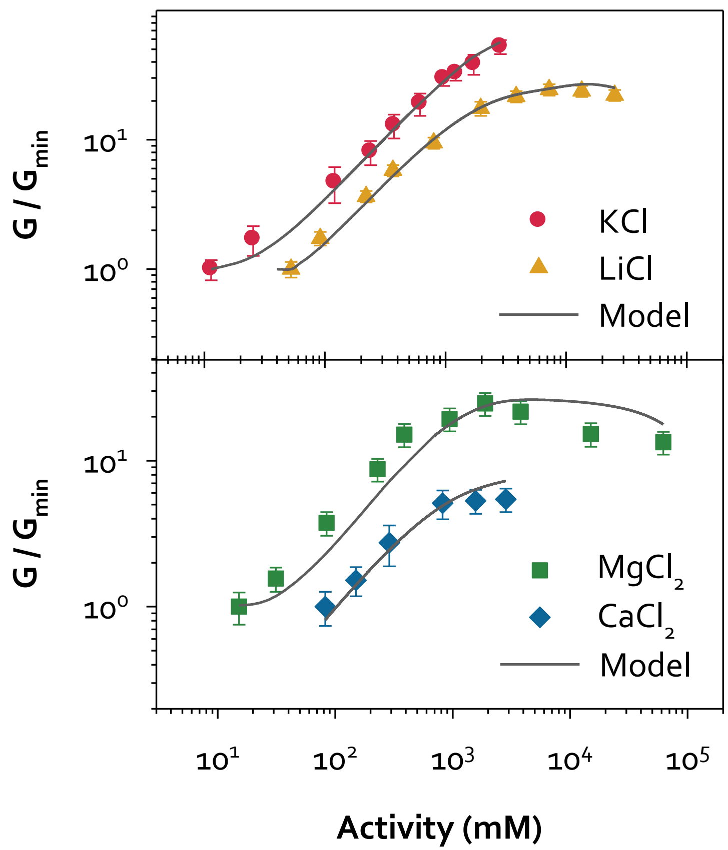




Exploring different conditions

Similar pattern for all salts under study & Two characteristic transport regimes

Conductance



Theoretical model

Channel fixed charges control ion transport

Donnan Equilibrium Equations

$$a_+ = -\frac{X}{2} + \sqrt{\left(\frac{X}{2}\right)^2 + a^2} \qquad a_- = \frac{X}{2} + \sqrt{\left(\frac{X}{2}\right)^2 + a^2}$$

$$G \sim z_+^2 D_+(a) \frac{a_+}{1 + a/K_d} + z_-^2 D_-(a) a_-$$

Existence of a binding site for cations

Langmuir Adsorption Isotherm

Exploring different conditions

Similar pattern for all salts under study & Two characteristic transport regimes

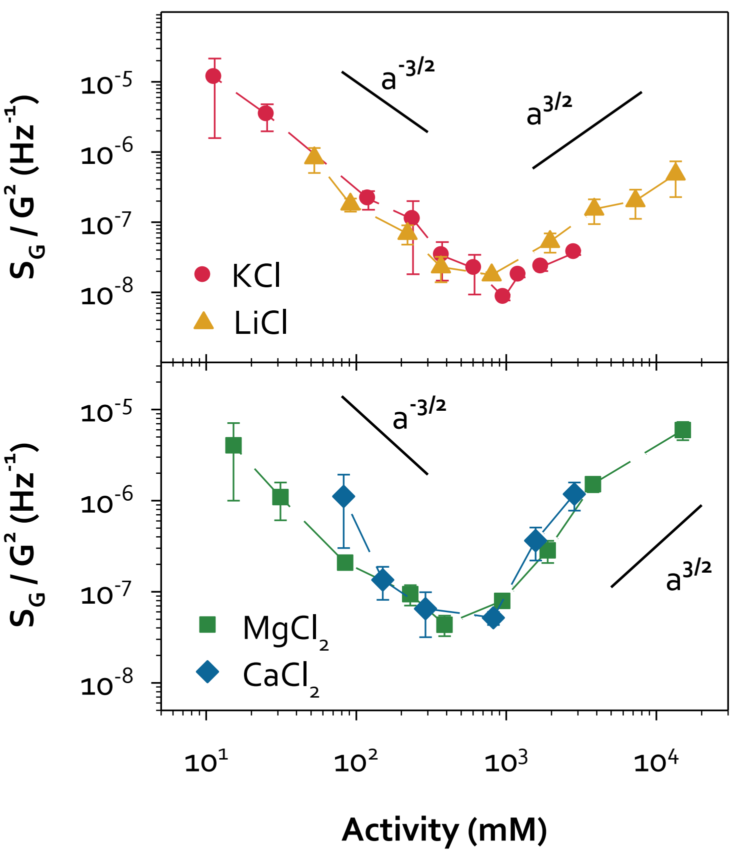
Conductance fluctuations S_G:

$$S_G = \left(\frac{S_G}{G^2}\right) \cdot G^2$$

Parabolic coefficient

Conductance

Parabolic coefficient



Exploring different conditions

Similar pattern for all salts under study & Two characteristic transport regimes

Low activity regime

$S_g \sim a^0$

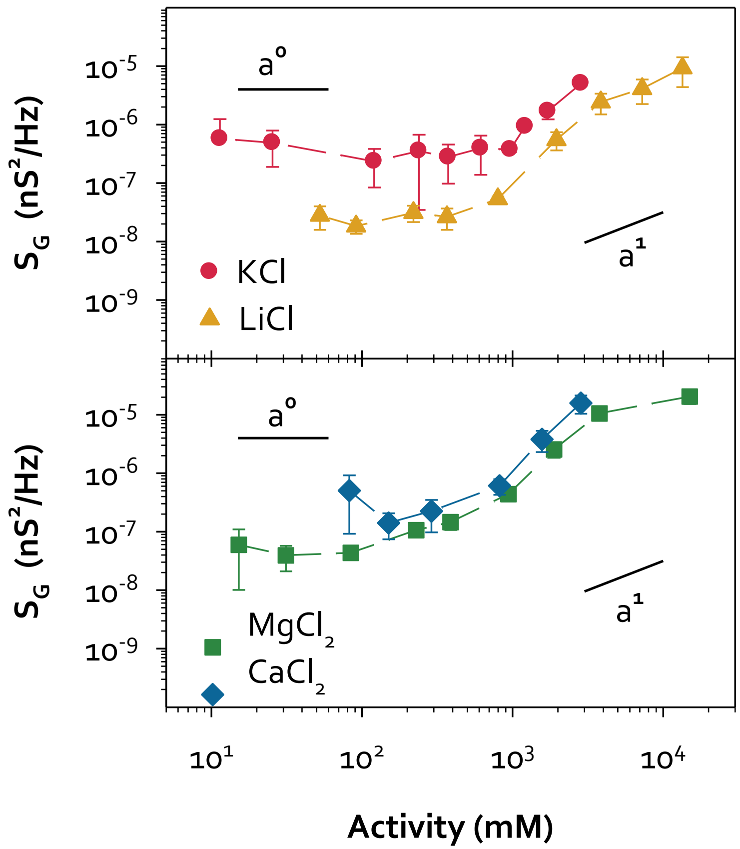
Controlled by channel fixed charges

High activity regime

$S_g \sim a^1$

Binding of cations increases the fluctuating particles at the pore walls

Conductance fluctuations



Summarizing…

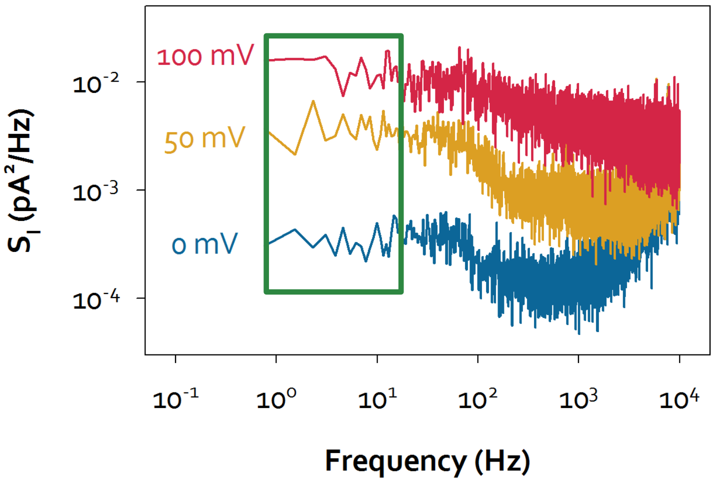
Use of a complimentary approach for the analysis of current fluctuations → Extract information from the PSDs without knowing much about the sources of noise

Overall view of the transport mechanisms of the channel

Functional evidence of the existence of a binding site for cations

But…

- We only take into account a part of the spectrum
- We do not extract any characteristic time from the PSDs



Acknowledgements

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Department of Physics

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Turning Discovery Into Health

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Thanks for your attention!

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