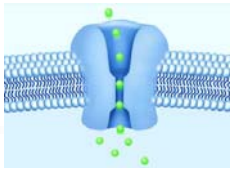

A Simulative Model for the Analysis of Conduction Properties of Ion Channels Based on First-Principle Approaches

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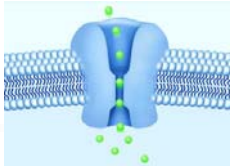
Outline



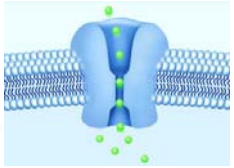
- The problem and our goal
- State of the art
- Our model and simulative procedure
- Results
- Further research developments

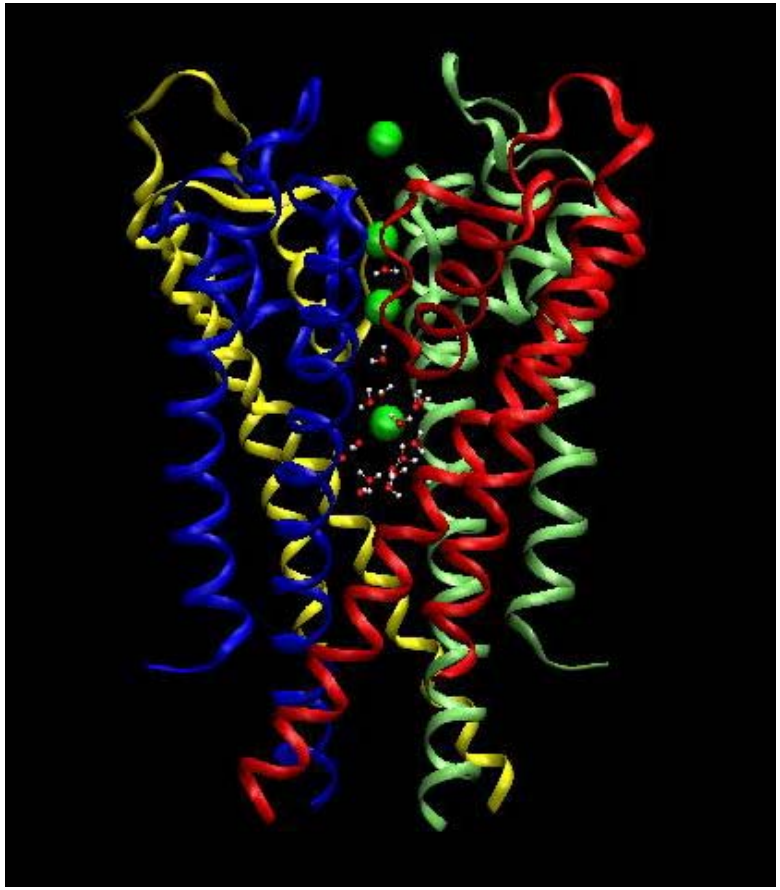
Ideas and hints also from:

M. Cascella, M. Ferrario, J. Kona,
S. Moroni, L. Reggiani, B. Roux, V. Torre

Supported by S3 and Italian Ministry of Education and Scientific Research

The problem...





VMD snapshot

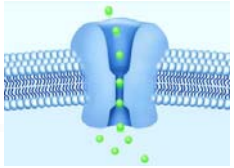
- Ion channels are nanometric macromolecular pores in cell membranes formed by proteins

- They have selective ion conduction and the ability to gate-open in response to an appropriate stimulus

- Since 1998 X-ray crystallographic structures, provinding atomic resolution of some channel proteins, have been obtained

- KcsA potassium channel is a good prototype to test any realistic simulative model

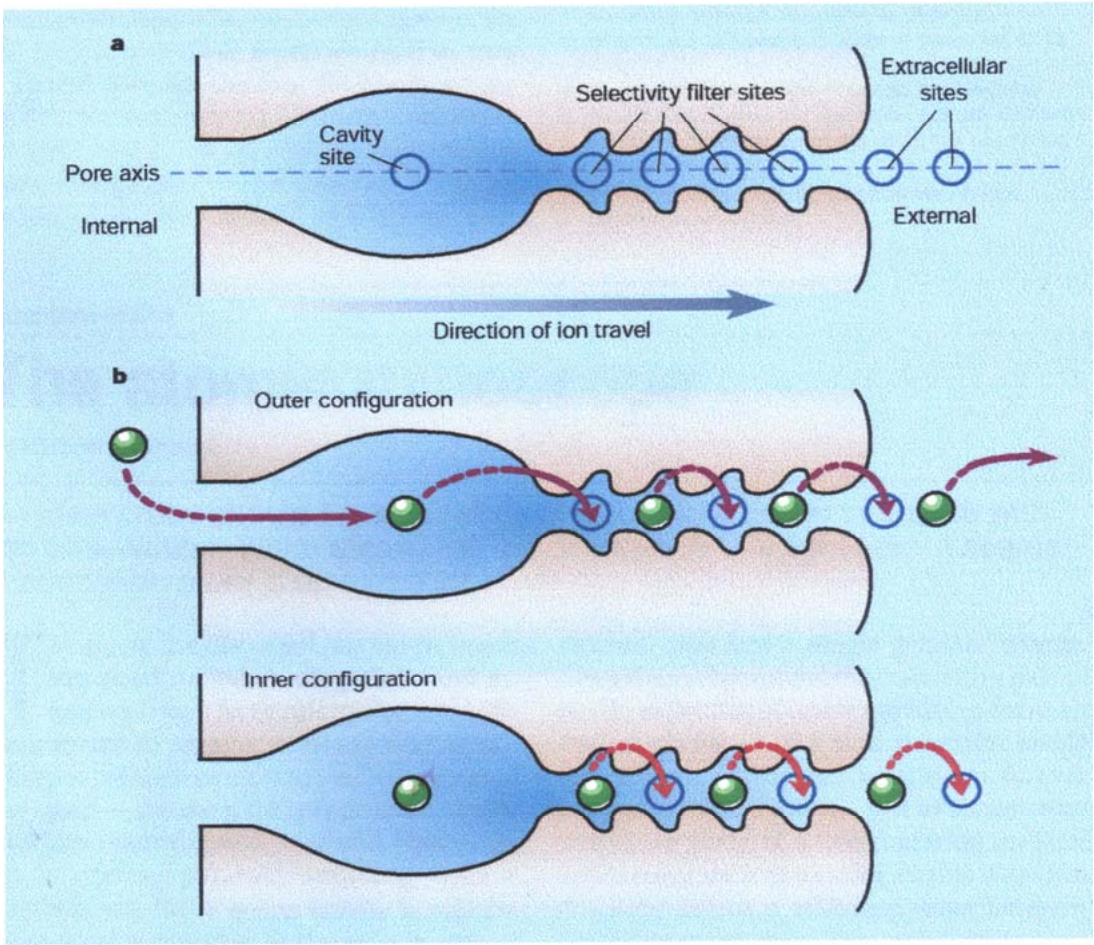
... and our goal



Molecular dynamics of single open ion channel

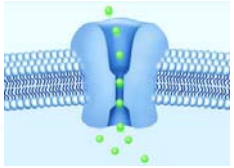
Monte Carlo simulation of multi-ion model

Conduction properties



From C. Miller, Nature, **414** *(2001)*

State of the art



Experimental scenario

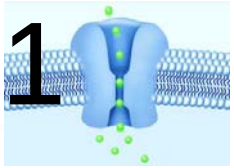
Electrical characterization of single open channels embedded in planar lipid bilayers in presence of buffered solutions with symmetrical K+ concentration

(see Miller – USA, Schrempf – Germany)

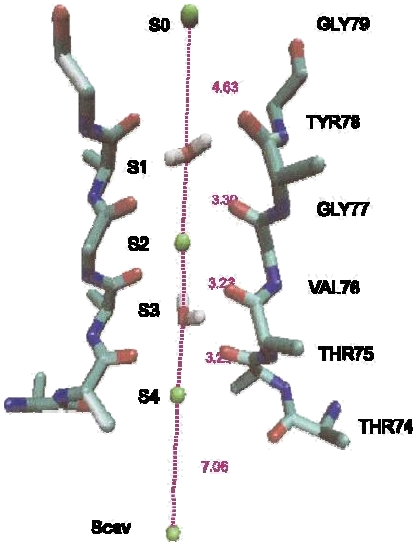
Theoretical models

- ✓ Single-file multi-ion models (e.g. Hille) (since 1970)
- ✓ Continuum Models, e.g. Poisson-Nernst-Planck (PNP) models (e.g. Heisenberg) (since 1970)
- ✓ Atomistic MD simulations and Brownian-Dynamics simulations (e.g. Roux) (since 1988)

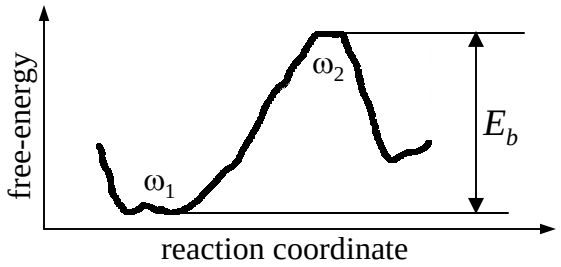
Our simulative procedure... 1



The possible configurations of the channel are defined through the K+ occupancy of a set of individual binding sites within the pore (S_{ext}, S_0...S_4, S_{cav}), identified from structural data and MD simulations, under the hypothesis of single-file concerted motion



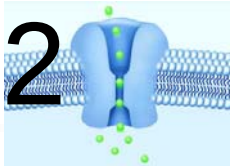
The transition rates can be estimated by calculating the average time a given transition needs to take place from MD or using the Kramers' formula:



$$k_{A\to B} = \frac{M\omega_1\omega_2}{2\pi D} e^{-\frac{E_b}{kT}}$$

where the diffusion coefficient D, the energy barrier E_b and the frequency ω_1, ω_2 come directly from MD simulations

Our simulative procedure... 2



The MD approach includes a modified GROMOS87 force-field. The high-resolution (2.0 Å) KcsA protein structure is embedded into a water-octane-water bilayer. A total of 34434 atoms have been considered.

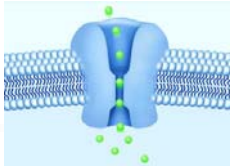
The free-energy difference between the initial and final configurations is evaluated from MD through a multiple-steering dynamics procedure

The ion capture probability assumes an energy barrier of 8 $k_B T$ (approx. 5 Kcal/mol) both at intracellular and extracellular reservoirs and has been estimated from the classical kinetic theory of gases:

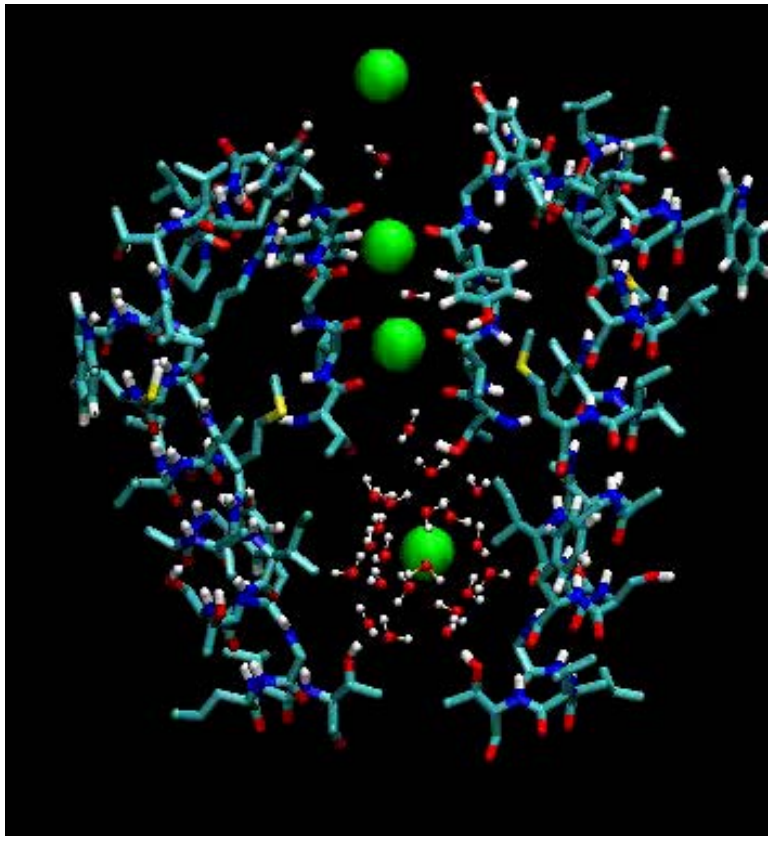
$$k_{\text{entry}} = \frac{n\sigma}{6}\sqrt{\frac{k_B T}{2M}}e^{-\frac{E_{in}}{kT}}$$

Being n the ion concentration and σ the cross sectional area of the vestibule

Results

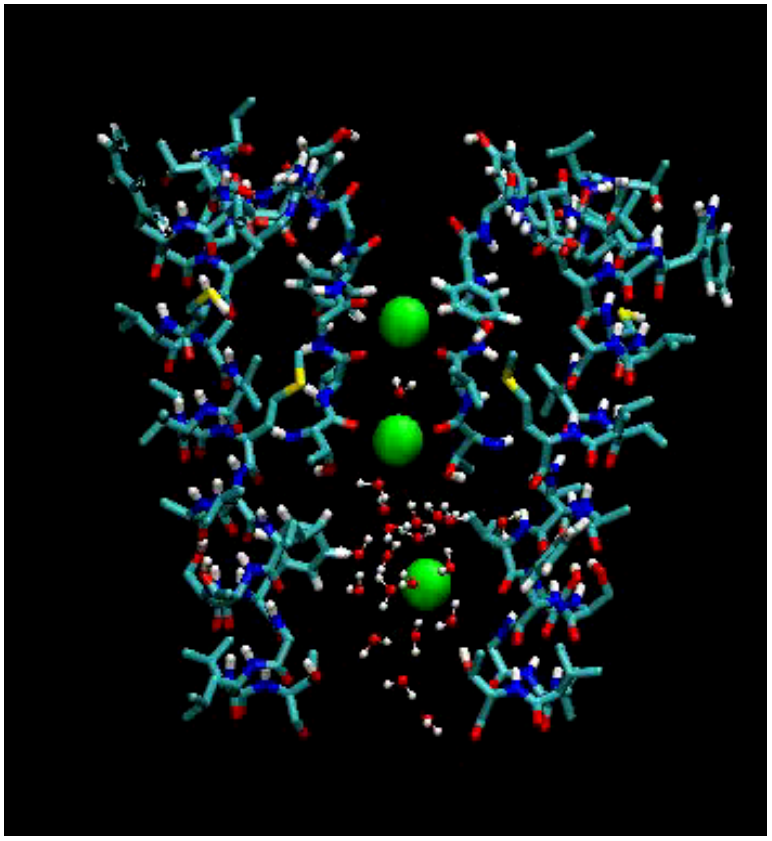


Observed transitions



VMD movie

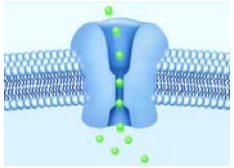
$(S_{ext},S_1,S_3,S_{cav}) \rightarrow (S_1,S_3,S_{cav}) \rightarrow (S_1,S_3,S_4)$



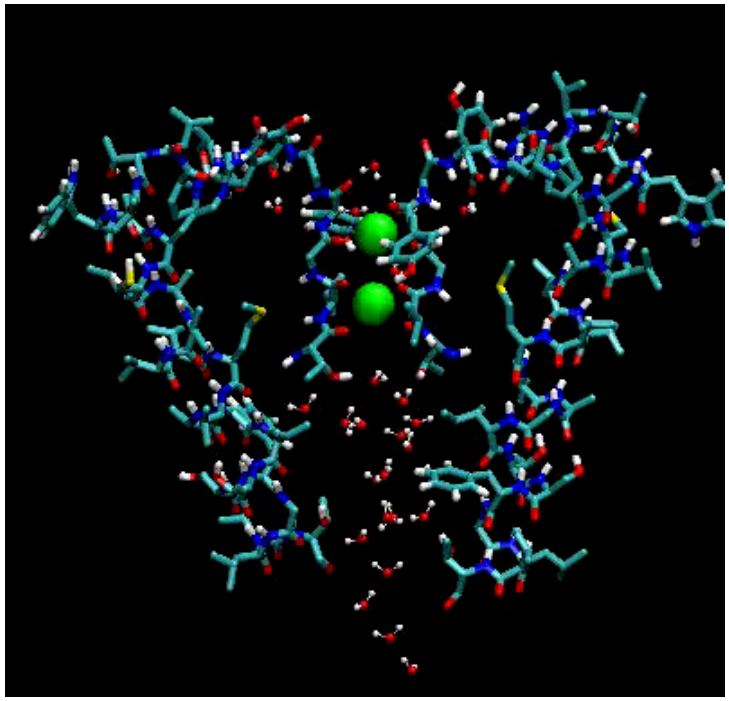
VMD movie

$(S_2,S_4,S_{cav}) \rightarrow (S_1,S_3,S_{cav}) \rightarrow (S_0,S_3,S_{cav})$

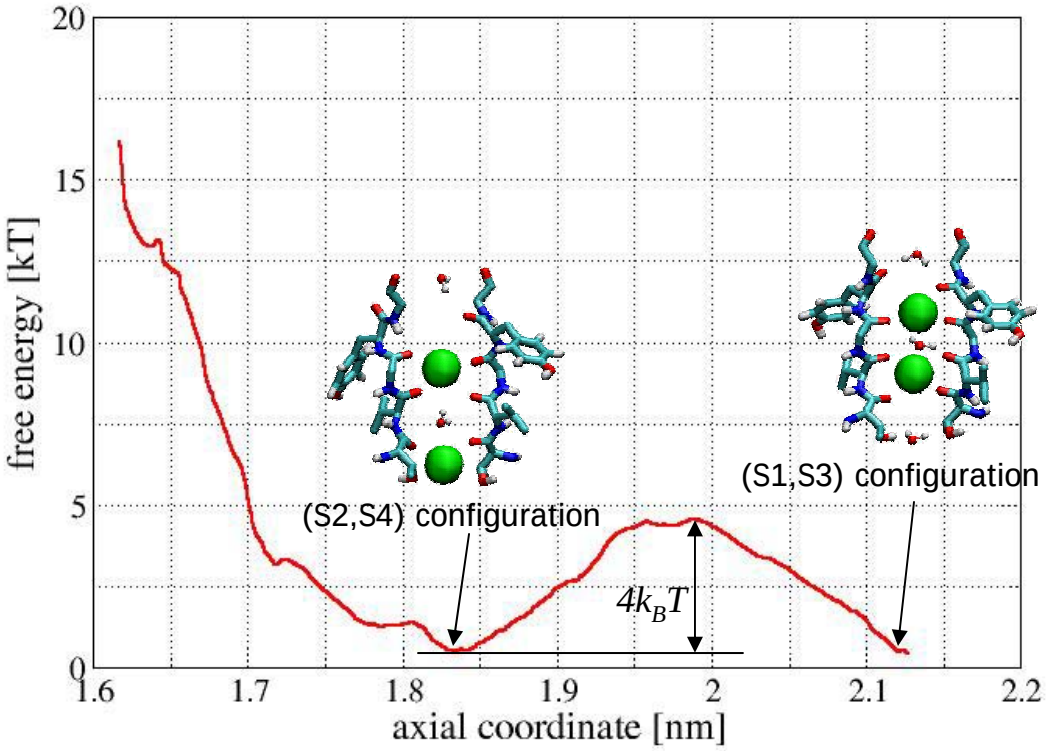
Results



Free-energy profile determination

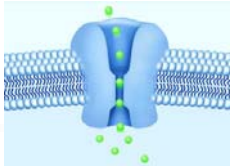


VMD movie

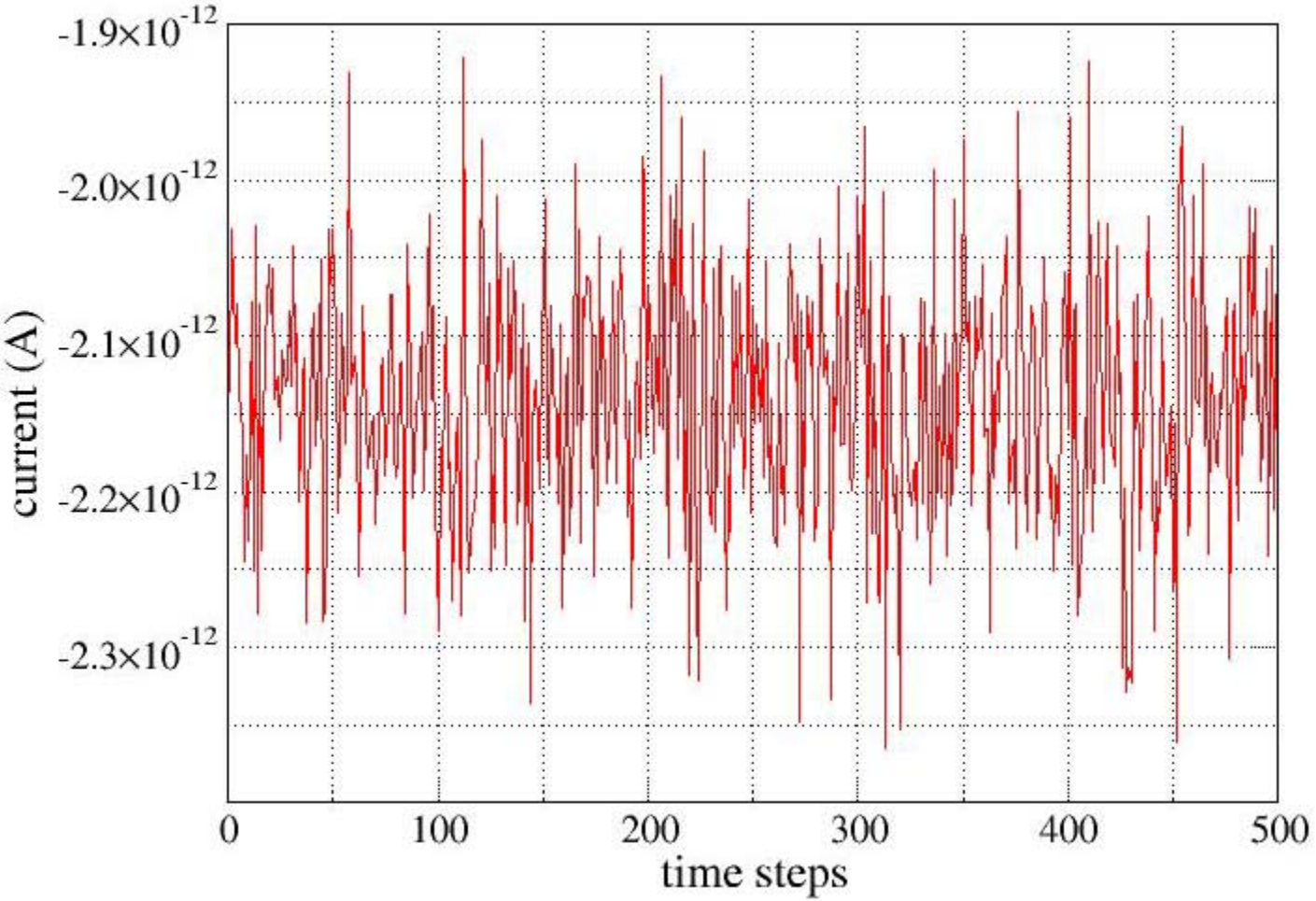


$(S_1,S_3) \rightarrow (S_2,S_4)$

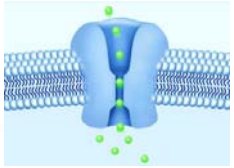
Results



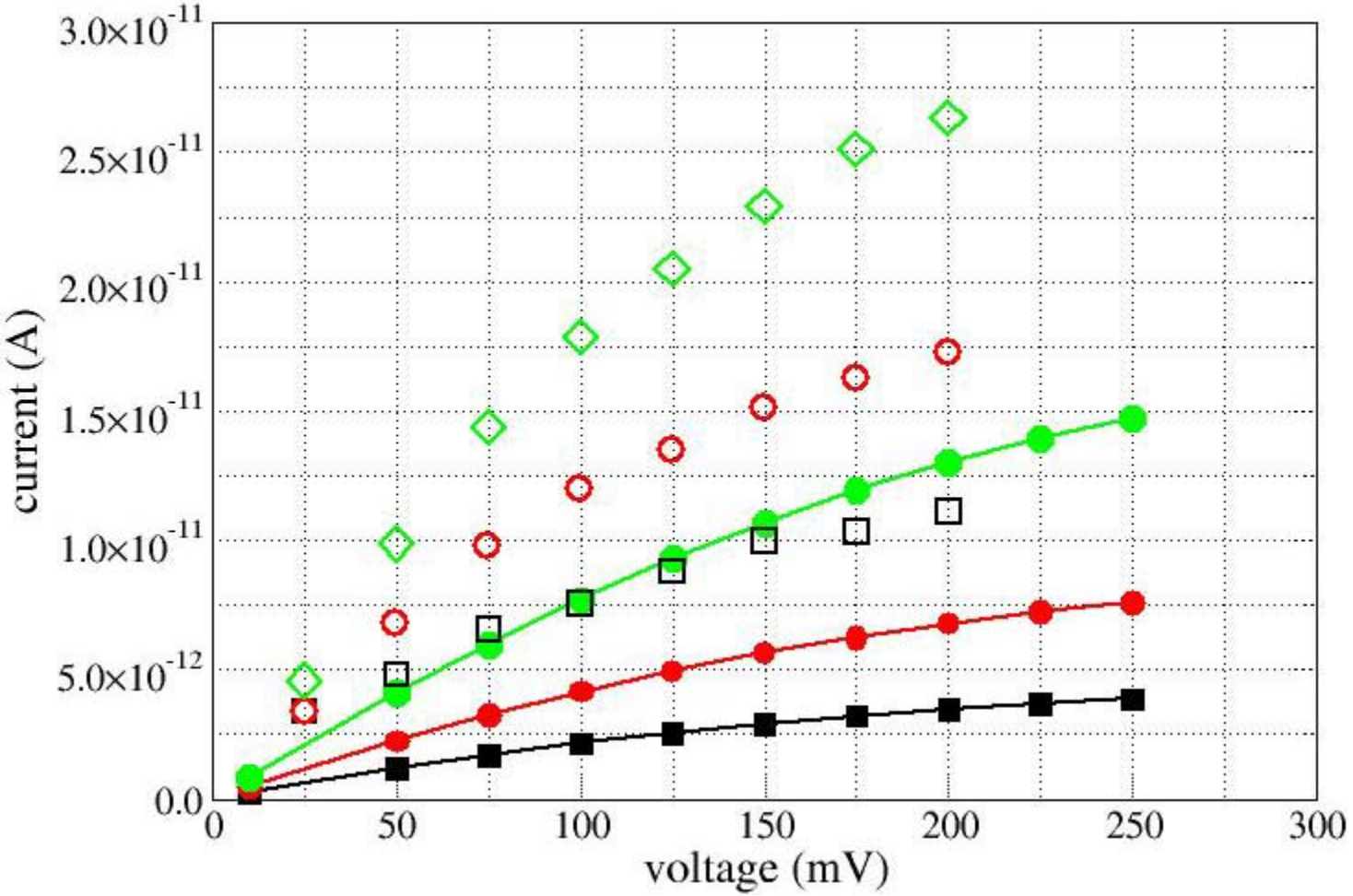
Current record from MC simulation



Results



I-V characteristics



K^+ concentration in reservoirs:

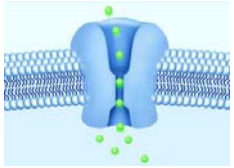
Black → 100mM

Red → 200mM

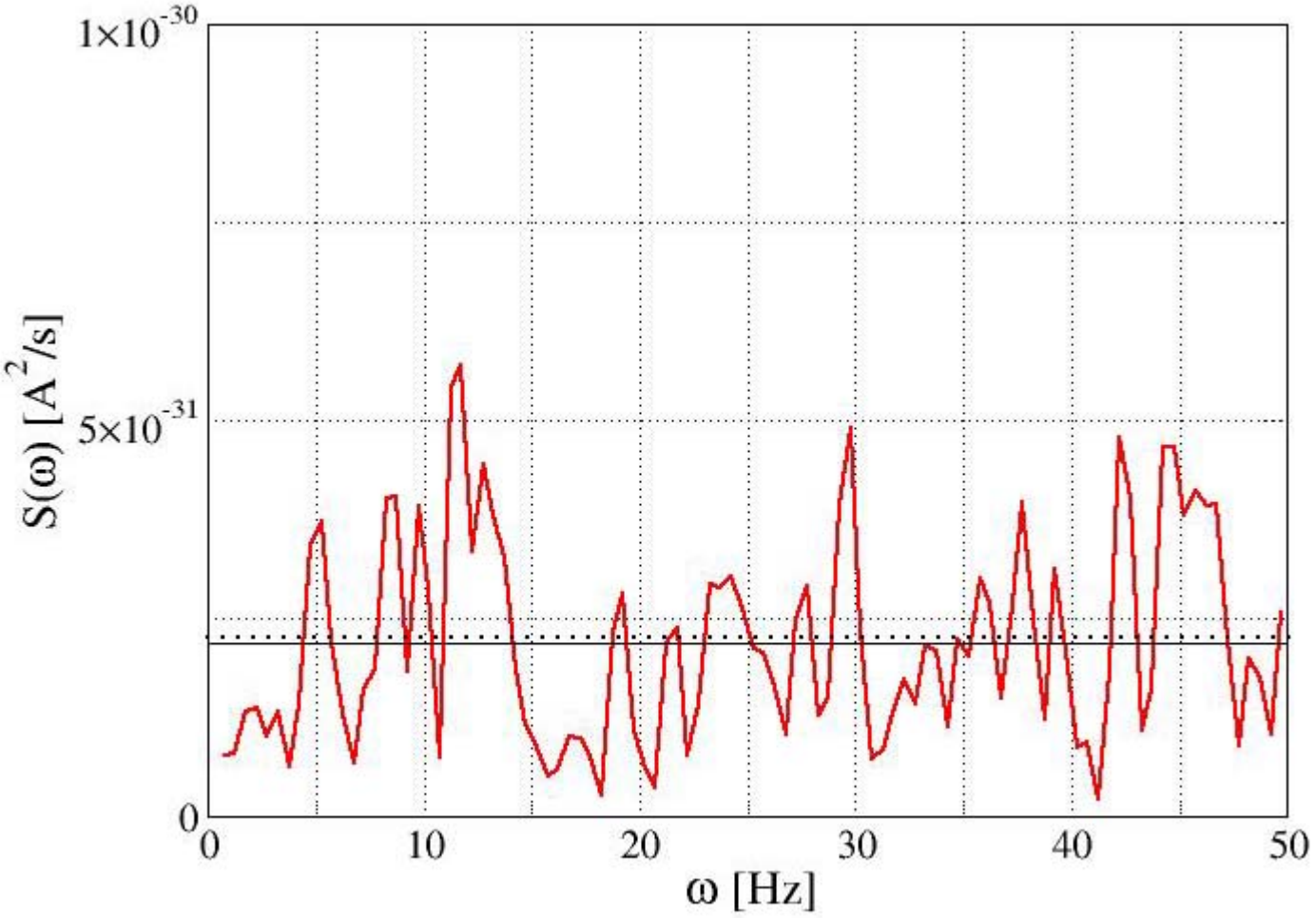
Green → 400 mM

Experimental data from LeMasurier et al., *J. Gen. Physiol.*, ***118*** *(3), 303 (2001)*

Results



Noise Power Spectrum



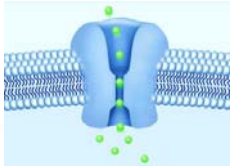
Theoretical value (dots)

$2.27 \cdot 10^{-31}$ A^2/s

Calculated average (line)

$2.18 \pm 0.13 \cdot 10^{-31}$ A^2/s

Further developments



- ✓ Free-energy mapping for all the relevant conduction paths
- ✓ Analysis of noise to identify possible correlations in ion exit
- ✓ Analysis of conduction properties in presence of gating
- ✓ Analysis of permeation properties of several group I ions (K, Na, Rb, ...)
- ✓ Analysis of other ion channels, with available structural conformation