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New insights in the translocation mechanism of ternary complexes of fluoroquinolones in E. coli

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Fluoroquinolones (FQs) are antibiotics with a large spectrum of action against Gram (-) and some Gram (+) bacteria. To penetrate the bacterial outer membrane across pores or through lipid/protein interface, OmpF is the main porin involved in the translocation of FQs in Gram(-) bacteria. Nevertheless, the misuse and overuse of antibiotics have triggered the development of bacterial resistance mechanisms against these drugs, specially through the decrease of the membrane permeability. The complexation of FQs with divalent metal ions and phenanthrolines (phen) is a strategy to try to bypass this problem, as it forms stable complexes (metalloantibiotics) with different activity and a possible alternative influx pathway. Thus, we proceeded to study the interaction of several FQs and ternary complexes of copper(II)/FQ/phen with OmpF Escherichia coli total external protein lipoproteins, using fluorescence spectroscopy and surface plasmon resonance (SPR), under physiological conditions (T=37°C; pH 7.4). This study aims to evaluate the differences in the translocation of the FQs and the metalloantibiotics to try to understand if they could be a good choice to circumvent, at least, one of the resistance bacterial mechanisms used against FQs.

Membrane conductance of Rhodobacter sphaeroides and the input of F0F1-ATPase in its formation

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The constant cycle of pump and leak of protons across the membrane contributes significantly to metabolic rate, particularly H₂ production by phototrophic bacteria. Current work is an attempt to reveal the input of F₀F₁-ATPase in membrane conductivity of R. sphaeroides, isolated from Armenian mineral springs. Proton conductance of bacterial membrane was measured at pH = 7.2, in the room temperature. Pulse doses of HCl rapidly decreased the extracellular pH over time the decrease was partially compensated by proton flux across the membrane, alkalinization of medium was detected. Cells treated with F₀F₁-ATPase inhibitor DCCD behaved similarly. Based on an estimated cellular buffering capacity the proton conductance of R. sphaeroides cells was 15 nmol of H⁺ /s/pH unit/mg protein. Addition of DCCD lowered this value, suggesting the involvement of F₀F₁-ATPase. However, under the conditions described, the decrease of proton conductance in the presence of DCCD was less than 10%, which shows the involvement of other proton leakage pathways. The role of membrane conductance in coupling mechanisms and bioenergetics of R. sphaeroides suggests it as a tool to interfere the H₂ production by these bacteria.

Non-equilibrium conduction through an open narrow ion channel

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We present a new theory of conduction in biological ion channels, able to account for the extraordinary selectivity of the potassium channel which conducts K⁺ at the diffusion-limited rate (like an open hole) while obstructing other monovalent ions by up to 1000x [1]. It extends our equilibrium theory [2] to encompass non-equilibrium conditions, using master equations for steady-state occupancy probabilities of states in the channel, and takes account of electrostatic interactions, the difference between bulk and channel excess chemical potentials, the voltage drop between the bulk and the channel, and the bulk concentrations. It reproduces the Langmuir adsorption isotherm and Michaelis-Menten current saturation. Model predictions agree well with experimental data. The theory is also applicable to other narrow channels and to artificial nanopores.

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References

Real-time visualization of membrane nanopore formation by MACPF/CDC proteins

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Proteins of the MACPF/CDC (membrane attack complex percin/cholesterol dependent cytolyisn) superfamily are effectors in pathogen attack and vertebrate immune defence. Released as soluble monomers, they can bind and self-assemble on a target cell membrane to form large transmembrane pores. Such pores can directly lyse and kill target cells, or allow transport of additional toxins. To study the assembly pathways of these proteins, we acquired high-resolution snapshots of by electron microscopy, and resolved membrane pore formation in real time by in-situ atomic force microscopy. In our most recent work, we have elucidated mechanisms of pore formation by the MACPF protein percin, a key mediator of lymphocyte cytotoxicity, used by our immune system to kill virus infected and cancerous cells.

Our experiments reveal that the pore assembly proceeds via a short, membrane-bound preporre intermediate. These short oligomers can insert into the membrane and subsequently recruit additional prepore oligomers to grow the pore size. These results highlight the diversity of assembly pathways in membrane pore formation and provide molecular-scale insight into the mechanism of immune killing.