Monday 14:30 - 16:00 Poster Session A @ Kloostergang & Novicengang

OLD DOMINION UNIVERSITY

Quantitative Dynamics of Molecular Transport After Minimal Electroperturbation

Very Dissimilar Stories for Very Similar Molecules

Esin B. Sözer[1], C. Florencia Pocetti[2,3] , P. Thomas Vernier[1]

[1]Frank Reidy Research Center for Bioelectrics, Old Dominion University, Norfolk, VA USA

[2] Department of Bioengineering, Instituto Tecnológico de Buenos Aires, Argentina [3] Consejo Nacional de Investigaciones Científicas y Técnicas, Buenos Aires, Argentina

email: esozer@odu.edu

Frank Reidy Research Center for Bioelectrics

Abstract

Molecular transport of small molecules after electropermeabilization has been studied for decades by monitoring intracellular fluorescence from normally impermeant dyes [1-4]. In contrast to schemes for larger molecules like DNA, the widely accepted mechanism for the transport of small molecules is diffusion through electropores [5] that are structurally similar to those observed in molecular dynamics simulations [6]. This simple picture cannot explain significant features of electropermeabilization, like long permeabilization lifetimes in cells [2,4] and varying estimates of pore sizes [7-8].

Quantitative analysis of transport of anionic (calcein) and cationic (YO-PRO-1, propidium) fluorescent dyes of comparable molecular size after exposure to single and multiple 6 ns, 20 MV/m pulses suggests a significant role for the transmembrane voltage in the migration of charged small molecules across permeabilized cell membranes. To investigate this possibility we studied anionic and cationic small molecule transport kinetics after electropermeabilization, and we used the new fluorescent membrane potential indicator FluoVolt™ to monitor transmembrane voltage in cells after similar permeabilizing pulse exposures. Preliminary results are consistent with the hypothesis that membrane potential is an important driver of small molecule transport in permeabilized cells.

Materials and Methods

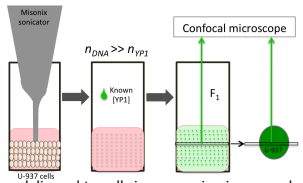
Cell lines and culture conditions: U-937 (human histiocytic lymphoma monocyte; ATCC CRL-1593.2) in RPMI-1640 culture medium containing 10% foetal calf serum (Gibco, Invitrogen), 100 U/ml penicillin and 100 µg/ml streptomycin at 37°C and 5% CO_2.

Fluorescence Calibration

A cell homogenate was prepared from dense U-937 cell suspensions (8×10^7 cells/mL) sonicated for two minutes with 0.1 % Triton-X100 (Misonix sonicator 4000). YO-PRO-1 or propidium were added in varying concentrations to the homogenate. Imaging was done with a Leica TCS SP8 confocal fluorescence microscope immediately after gentle stirring [9].

Molecular Transport Experiments

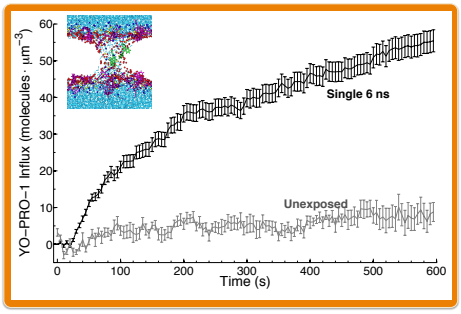
Cells were washed and suspended at approximately 5×10^5 cells/mL in fresh medium containing for influx experiments either 2 µM YO-PRO-1, 30 µM propidium or 200 µM calcein. For calcein efflux measurements cells were loaded with 0.5 µM calcein-AM for 30 minutes at 37 °C before washing and re-suspension in fresh medium.

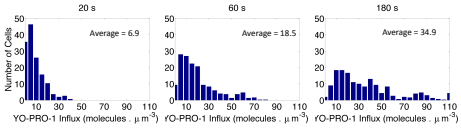


6 ns, 20 MV/m pulses (FID pulse generator FPG 10-10NK) were delivered to cells in suspension in cover glass chambers [Nunc™ Lab-Tek™ II] through parallel tungsten wire electrodes [10]. Cells were observed at laboratory room temperature on the stage of a Leica TCS SP8 laser scanning confocal microscope

Results

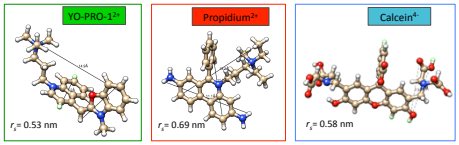
YO-PRO-1 Transport after Single 6 ns Pulse

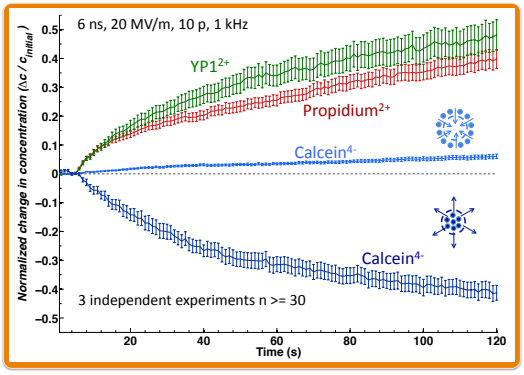


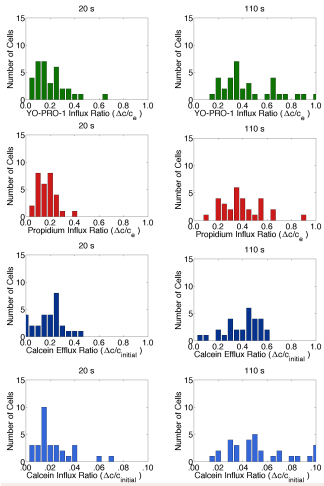


The mean molecular influx over the first 20 s after pulse exposure is 7 YP1 µm^{-3} · s^{-1}, or about 180 molecules per cell per second (assuming an average cell radius of 5 µm). The YP1 fluorescence increase is uniform, with no preferential point of entry into cells.

Small Molecule Transport after Multiple 6 ns Pulses



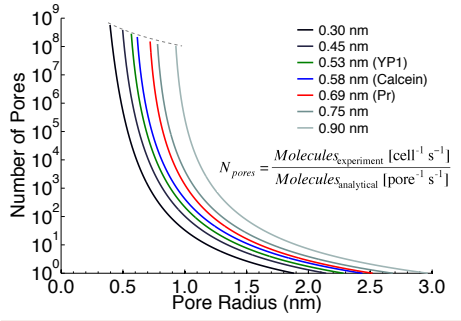




$\varphi_{i,YP1} > \varphi_{i,Pr} >> \varphi_{i,calcein}$ $\varphi_{e,calcein} >> \varphi_{i,calcein}$

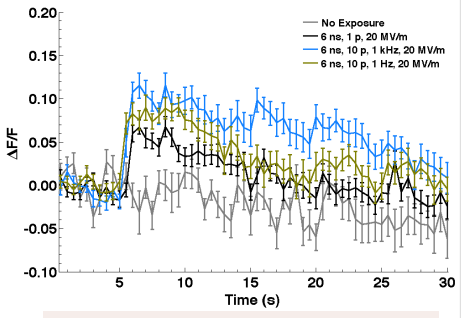
Transport is heterogeneous among the cell population for all dye molecules.

Diffusive Influx Calculations

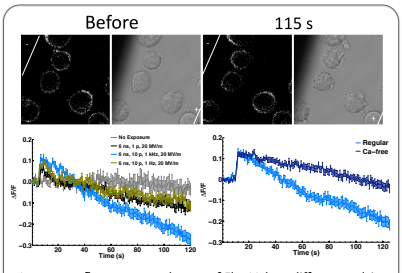


Very small changes in solute or pore size can modify the amount of diffusive flux by several orders of magnitude.

Membrane Potential Measurements



Depolarization of the membrane at the pulse exposure is observed as a jump in fluorescence.
The fluorescence recovers in ~25 seconds.



Long term fluorescence change of FluoVolt at different pulsing conditions might be related to membrane damage/repair mechanisms.

Since calcium is known to play a crucial role in membrane repair, the same experiments were performed in calcium-free medium. The long term behavior of FluoVolt fluorescence is different in the absence of calcium.