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Supporting material

Title: ROUTES OF EPITHELIAL WATER FLOW: AQUAPORINS VERSUS COTRANSPORTERS

Authors: Rustam Mollajew, Florian Zocher, Andreas Horner, Burkhard Wiesner, Enno Klussmann, and Peter Pohl

Correction of the diffusion constants for increased viscosity

The increased viscosity in the hyperosmotic compartment resulted in a decrease of D_{Mg}. Thus, determination of v by fitting Eq. (9) to the experimental concentration profiles required knowledge of D_{Mg} at any of the PEG concentrations used. Therefore we measured the electrical conductance of a $MgCl_2$ solution at different PEG concentrations with a conductometer and assumed that the conductivity ratio in the absence and presence of PEG is equal to the ratio of the respective diffusion constants.

Since the increased viscosity should have a larger effect on D_P than on D_{Mg}, we exploited fluorescence correlation spectroscopy (FCS) to estimate D_P. In brief, the average residence time τ_D of single fluorescent molecules (rhodamine 6G) in the focal volume was derived from the autocorrelation function $G(\tau)$ of the fluorescence temporal signal which was acquired by a commercial laser scanning microscope equipped with avalanche diodes (LSM 510 META ConfoCor 3, Karl Zeiss, Jena, Germany). A water drop formed the connection between the objective of the microscope and the cover slip, which provided the bottom of the measurement chamber. D was determined as $\omega^2/4\tau_D$ with an absolute accuracy of about 20 % (2). For the scope of the current work, only relative changes of D are important, and these were determined with a higher accuracy. The ratio D/D_0 of rhodamine diffusion coefficients at a certain PEG concentration and in its absence was determined at an accuracy which is limited by changes of the refractive index. Since at concentrations of up to ≤ 0.5 M, PEG did not augment the refractive index above 1.38, the error in determination of D/D_0 is < 12 % (1).

Reference List

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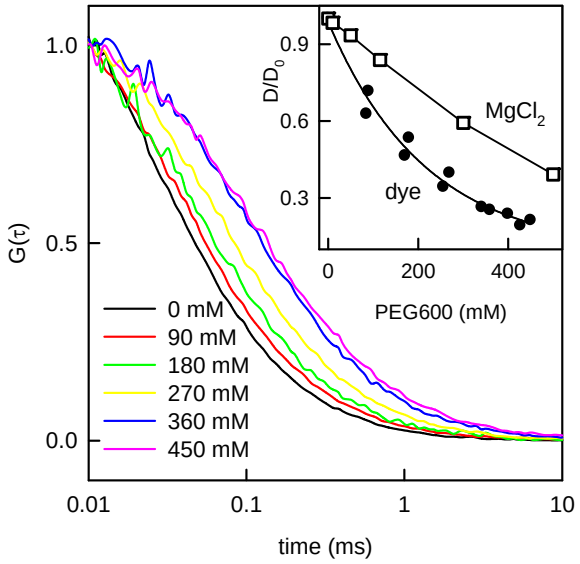
2. Przybylo, M., J. Sykora, J. Humpolickova, A. Benda, A. Zan, and M. Hof. 2006. Lipid Diffusion in Giant Unilamellar Vesicles Is More than 2 Times Faster than in Supported Phospholipid Bilayers under Identical Conditions. *Langmuir* 22:9096-9099.



Supplementary Figure 1: Effect of increased viscosity on solute mobility. Osmolyte addition caused an increase in viscosity. The decrease in PEG600 mobility was derived from fluorescence correlation spectroscopy measurements of rhodamine 6G mobility in solution. The shift of the autocorrelation functions of dye fluorescence intensity to longer times corresponds to a decrease in the dye's diffusion coefficient (D). The effect is shown in terms of the ratio of D in the presence of PEG600 and D_0 measured in its absence (inset). For the smaller Mg^{2+} ions a weaker dependence of mobility on viscosity is expected. D/D_0 for Mg^{2+} was derived from conductivity measurements in a buffered 0.1 M $MgCl_2$ solution (inset).