Quantification of Spectroscopically Resolved Glycerol and Water Exchange in Human Aquaporins with Diffusion Exchange Methods

S.E. Mailhiot, S. de Mare, K. Lindkvist-Petersson, D. Topgaard

Lund University, Lund, Sweden

Aquaporins are cellular membrane proteins that transport water. They are critical for maintaining a stable osmotic pressure within the cell. A subset of aquaporin proteins also transport non-charged molecules such as ammonia, glycerol, and carbon dioxide. NMR is well suited for measuring membrane permeability. Previously, it has been shown that NMR diffusion exchange methods (1-3) can be used to measure the exchange rate of water across the cellular membrane, but for membrane proteins that co-transport molecules more information is needed. This work expands on previous filter exchange spectroscopy (FEXSY) methods (2,3). Specifically, we present a method for the simultaneous measurement of glycerol and water transport in yeast cells with and without human aquaporins. The goal of this work is to quantify the ratio of water to glycerol molecules transported by the aquaporin using the exchange times and mass transport equations.

The method proposed here uses a double pulsed gradient stimulated echo (PGStE) with variable mixing and echo times, τ_m and τ_e respectively, and records the free induction decay. The double PGStE is used to accommodate samples with short T_2 relaxation times. The variable echo spacing combines FEXSY with a D-T_2 measurement (3). Finally, the free induction decay and consequently the chemical spectra is acquired. Using spectral resolution and peak selection, the exchange of water and glycerol is measured. The pulse program and a data set are shown below. Yeast cells expressing aquaporin in culture medium and 15% glycerol were studied. An exchange life time of 0.2 and 2.0 seconds is measured for water and glycerol, respectively. The advantage of this method is the ability to measure exchange with spectral resolution. The non-invasive measurement of exchange across the cellular membrane can be used to understand properties of the aquaporin transporter, specifically the co-transport of water and glycerol.

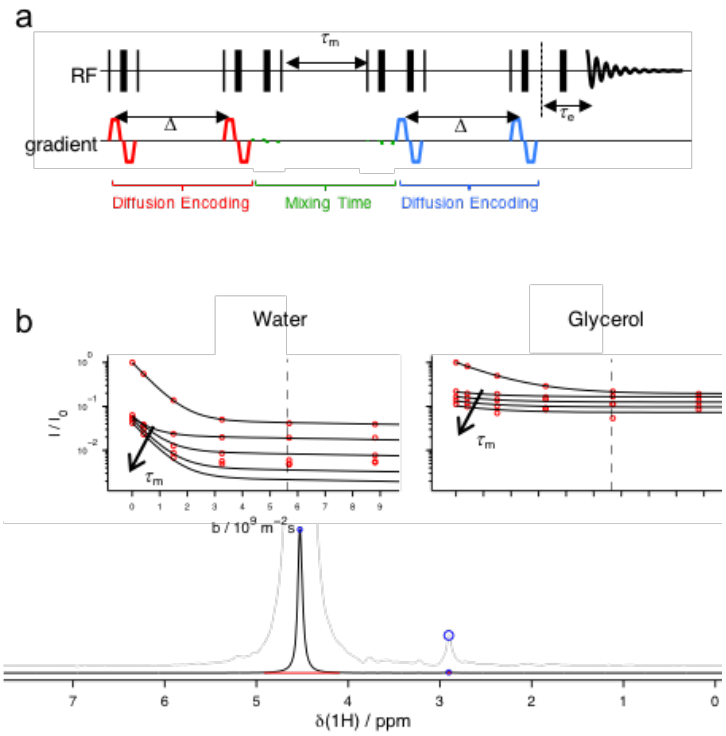

Figure 1- (a)The pulse program is composed of an initial diffusion encoding period (red), a mixing period with mixing time τ_m where molecules diffuse between the intracellular and extracellular spaces (green), diffusion encoding where the gradient amplitude is varied (blue), and a variable echo time τ_e. A thin radio frequency (RF) line represents a 90^0 pulse and a thick line represents a 180^0 pulse. The free induction decay is aquired. (b) A data set for one echo time is displayed. The signal decay is shown for water at 4.53 ppm and glycerol at 2.9 ppm. The top line in the signal decay is obtained without a diffusion filter and the lines below use a diffusion filer and short to long mixing times from top to bottom. An exchange life time of 0.2 and 2.0 seconds is measured for water and glycerol, respectively.

References

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