Activation Energies for Water Diffusion in ex-vivo White Matter

B. Dhital1, C. Labadie1,2, H. E. Müller1, and R. Turner1

1Max Planck Institute for Human Cognitive and Brain Sciences, Leipzig, Germany, 2Laboratoire de Spectrométrie Ionique et Moléculaire, Université Claude Bernard Lyon 1, France

Introduction: Diffusion in brain tissue is well known to be non-Gaussian, resulting in major efforts to understand the biophysical basis of water mobility in such tissues [1]. This study aims to understand the energetics of differently diffusing components by quantification of their respective activation energies. When temperature is varied, self-diffusion in pure liquids follows the Arrhenius law, \( D(T) = D_0 \exp(-E_a/RT) \), where \( E_a \) is the activation energy for diffusion \( D(T) \), and \( R \) is the universal gas constant [2]. This equation can be fitted for each diffusion component.

Methods: One small excised block of formalin-fixed human corpus callosum (8 mm diameter, 7 mm height) was placed in a NMR tube, with the prevailing axonal orientation parallel to the main magnetic field of a homebuilt Fegris NT 125 MHz spectrometer [3]. The ex-vivo study was approved by the local ethics committee. The spectrometer is equipped with a unidirectional ultra-high gradient system, with peak gradient strength of 35 T/m parallel to the main magnetic field. Temperature was controlled and monitored within 0.1 °C by means of a liquid nitrogen supply and built-in heater/thermometer system. The temperature was gradually decreased from 20 °C to -23 °C at 1 °C intervals. A control spin echo acquisition was performed at each temperature (\( TE = 2.4 \text{ ms} \)). At about 5 °C intervals, water self-diffusion was measured using the Stejskal-Tanner sequence (\( TE = 2.4 \text{ ms}, TR = 2.5 \text{ s} \)) [4]. To minimize effects of exchange between water compartments, the diffusion time was kept very short (\( J = 1.2 \text{ ms} \)) and the diffusion gradient duration (\( \delta = 0.5 \text{ ms} \)). The attenuation plots at each temperature were fitted to a bi-exponential function \( S(b) = S_0 (P_f \exp(-bD_f)) + P_s \exp(-bD_s)) \), using the Matlab® robust regression (robustfit). The diffusion coefficients thus obtained were logarithmically plotted against reciprocal of temperature, yielding a slope of \(-E_a/R\) for each [1].

Results: Figure 1 shows the signal attenuation at different \( b \)-values, and the corresponding bi-exponential fits. At 20 °C, \( D_f = 0.69 \mu m/\text{ms} \), \( D_s = 0.07 \mu m/\text{ms} \) and \( P_s = 0.42 \), while at -23 °C \( D_f = 0.13 \mu m/\text{ms} \), \( D_s = 0.04 \mu m/\text{ms} \) and \( P_s = 0.89 \). Figure 2 shows a sharp drop in signal at -20 °C where almost all of the fast pool and more than half of the slow pool underwent a phase transition from liquid to solid state [5] and became no longer observable at the echo time of 2.4 ms [6]. The signal from the remaining mobile water showed mono-exponential dependence on \( b \)-factor, with a low \( P_s \) (Figure 2) and \( D_f \) (Figure 3). The Arrhenius fit of \( D \) against 1/\( T \) (Figure 3) gave a mean activation energy for the fast pool of 13.2 ± 0.8 kJ/mol, reasonably close to the value of 19.2 kJ/mol for pure water [2]. For the slower pool, \( E_a \) was found to be much smaller, 6.5±1.0 kJ/mol. Extrapolation of the results for the two pools to human body temperature (37°C) gave estimated values of 0.96 \( \mu m/\text{ms} \) (fast) and 0.086 \( \mu m/\text{ms} \) (slow) respectively, which are reasonably consistent with corresponding in-vivo results [1].

Conclusion: Observation at the ultra-short diffusion time of 1.2 ms of two distinct pools of mobile water within ex-vivo white matter, with activation energies differing by a factor of two, provides clear evidence that the non-Gaussian diffusion signal indeed arises from separate water compartments, and is not merely an effect of restriction within a single compartment by cell membranes. Only about half the slow component freezes out in the same way as bulk water, suggesting that the slow diffusion pool is itself a two-component system, in very fast exchange compared with the diffusion time \( J \) used in this study. The unfrozen water [5] fraction within the slow diffusion pool may be strongly interacting with membranes and macromolecules [5,7]. The activation energy of the slow pool (\( E_a = 6.5±1.0 \text{ kJ/mol} \)) corresponds quite well to the energy required to break a hydrogen bond in a locally structured domain [8]. A decrease in diffusion has also been reported to accompany a drop in activation energy in the formation of lipid micro-domains in membranes containing sphingomyelin [9]. The unfrozen water pool observed in this study may thus consist of Debye layers of hydrated water close to intracellular membranes [1].


Fig. 1: Effect of temperature on the diffusion signal attenuation for excised, formalin-fixed ex-vivo human corpus callosum. Data were fitted with a bi-exponential function describing fast and slow diffusing components.

Fig. 2: Comparison of normalized spin echo signal (SE) with the total water, slow and fast signals (\( S_0 \), \( S_0P_f \), \( S_0P_s \)) obtained from the bi-exponential fit.

Fig. 3: Arrhenius plot of diffusion coefficients. At phase transition (~20°C) the slow and fast diffusion are ill-defined. Below this temperature the signal attenuation may be described with a mono-exponential (not shown).