Activation Energies for Water Diffusion in *ex-vivo* White Matter

B. Dhital[1], C. Labadie[1,2], H. E. Möller[1], and R. Turner[1]
[1]Max Planck Institute for Human Cognitive and Brain Sciences, Leipzig, Germany, [2]Laboratoire 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 ms). At about 5 °C intervals, water self-diffusion was measured using the Stejskal-Tanner sequence (TE = 2.4 ms, TR = 2.5 s) [4]. To minimize effects of exchange between water compartments, the diffusion time was kept very short (Δ = 1.2 ms) and the diffusion gradient duration (δ = 0.5 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 (*robusfit*). The diffusion coefficients thus obtained were logarithmically plotted against reciprocal of temperature, yielding a slope of $-E_a/R$ for each [1].

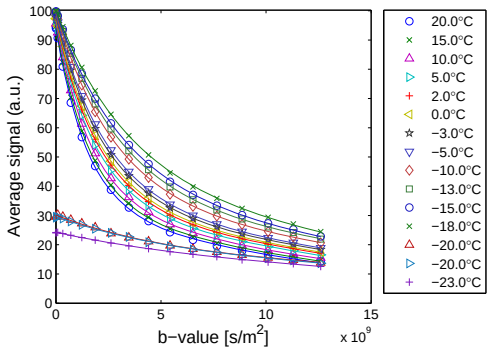

Fig. 1: Effect of temperature on the diffusion signal attenuation for excised, formalin-fixed *ex-vivo* human corpus callosum. Data were fitted with a bi-exponential function describing fast and slow diffusing components.

Results: Figure 1 shows the signal attenuation at different b-values, and the corresponding bi-exponential fits. At 20°C, D_f = 0.69 µm^2/ms, D_s = 0.07 µm^2/ms and P_s = 0.42, while at -23 °C D_f = 0.13 µm^2/ms, D_s = 0.04 µm^2/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_f (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 µm^2/ms (fast) and 0.086 µm^2/ms (slow) respectively, which are reasonably consistent with corresponding in-vivo results [1].

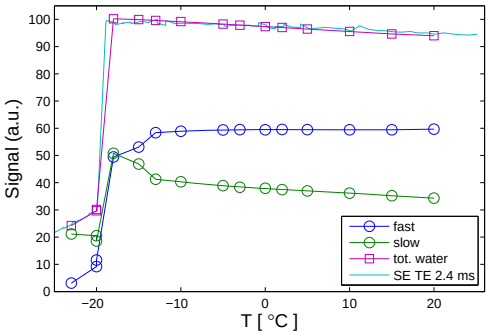

Fig. 2: Comparison of normalized spin echo signal (SE) with the total water, slow and fast signals (S_0, $S_0 \times P_f$, $S_0 \times P_s$) obtained from the bi-exponential fit.

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 Δ 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 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].

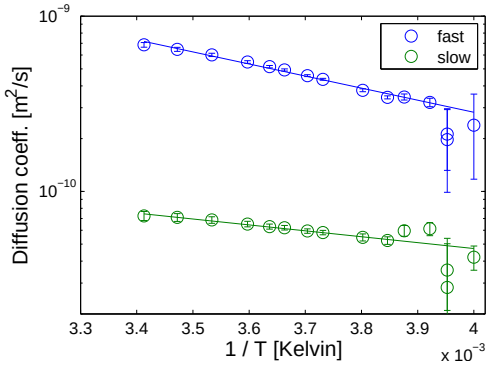

Fig. 3: Arrhenius plot of diffusion coefficients. At phase transition (-20°C) the slow and fast diffusion are ill-defined. Below this temperature the signal attenuation may be described with a mono-exponential (not shown).

References: [1] Le Bihan 2007 *Physics Med. Biol.* 52:R57. [2] Kausik 2009 *JACS* 131:18254. [3] Galvosas et al. 2001 *J. Magn. Reson.* 151:260. [4] Stejskal & Tanner. 1965 *J. Chem. Phys.* 42:288. [5] Wolfe et al. 2002 *CryoLetters* 23:157. [6] Escanyé et al. 1984 *J. Magn. Reson.* 58:118. [7] Pal et al. 2002 *PNAS* 99:1763. [8] Smith et al. 2006 *Science* 306:851. [9] Filippov et al. 2006 *Biophys. J.* 90:2086.