Probing displacements within and exchange among tissue microenvironments using static gradient spin echo diffusion and DEXSY NMR

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Introduction: In systems with microscopic fluid compartments that communicate on timescales similar to the diffusion encoding time, resolution of pulsed gradient spin echo (PGSE) NMR is limited by the maximum gradient strength and the gradient pulse duration. Spin echoes acquired in a large static magnetic field gradient break this microstructural resolution limit and probe nanoscale structures in sub-millisecond timescales [1]. Under a static gradient g, the signal attenuation regime is determined by the restriction length l_s relative to the free diffusion length $l_D = \sqrt{2D_0\tau}$ and the dephasing length $l_g = (D_0/\gamma g)^{1/3}$ [2]. Diffusion EXchange SpectroscopY (DEXSY), both in a full [3] and a rapid approach [4], probes the exchange of water between these restricted environments. New methods to clear lipid membranes from tissue [5] provide a means to interrogate how water in distinct tissue microenvironments migrates within them and exchanges among them, providing a new window on tissue microstructure and microdynamics.

Methods: NMR measurements were performed at 13.79 MHz and g=15.3 T/m (l_g=800nm) on a PM-10 NMR MOUSE with a Kea2 spectrometer (Magritek). A double-wrapped solenoid RF coil (39 turns, 2mm ID, 1.3cm length) and circuit board were built in-house to maximize filling factor and SNR. The coil was glued within a chamber, specially built to maintain vitality of live specimens and to control temperature (7–37°C range). Experiments were performed on isolated spinal cords of newborn Swiss Webster wild type mice fixed in 4% paraformaldehyde. Static gradient spin echo 1-D diffusion (43 points, $\tau = 0.2 \rightarrow 6.55$ms) [6] and 2-D DEXSY ($21 \times 21$ points, $\tau_1, \tau_2 = 0.2 \rightarrow 3.3$ms, written in-house, 8-step phase cycle) experiments with CPMG acquisition (2000 echoes, TE=25µs, 400µm slice, TR=2s) were used to probe cellular and subcellular membrane components and water exchange between them.

Results and Discussion: About 25% of the signal arises from restricted water pools. About 5% of the signal is from restricted water with l<800nm. Water exchanges between restricted and free environments with a rate of roughly 100 1/s. Clearing lipids with 10%wt. Triton X surfactant shows membranes to be the origin of 99% of the water restriction.

Conclusion: Large static gradients provide a means to probe nano and microscale tissue components and the exchange between them. Exchange rates indicate that standard PGSE methods cannot resolve such components. The sensitivity of diffusion NMR to tissue microstructure is almost entirely due to membranes.

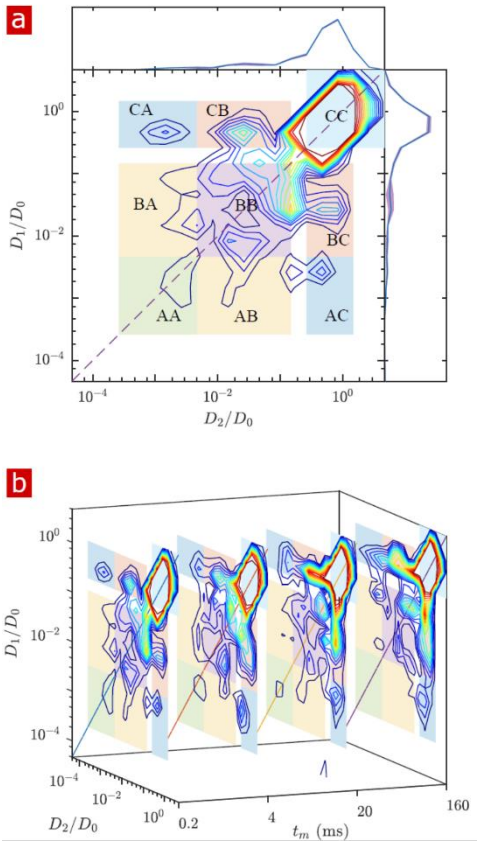

Fig. 1: DEXSY distributions for a fixed spinal cord sample (a) at mixing time t_m = 0.4ms and (b) at increasing t_m showing the build-up of exchange (off-diagonal) components and decay of the non-exchange (on-diagonal) components.

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