Double-PFG MR reveals insights into compartment shape, organization and morphology in heterogeneous specimens

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Introduction. Noninvasively measuring underlying microstructural features of opaque heterogeneous specimens is extremely important in many scientific disciplines ranging from characterization of porous media to neuroscience. Single-Pulse-Field-Gradient (s-PFG) diffusion MR has become the most important method for characterizing microstructure in white matter since it can detect diffusion anisotropy when fibers are coherently organized[1] (i.e. are characterized by ensemble anisotropy (eA)), and can even measure compartment size in such scenarios using the q-space approach[2]. However, s-PFG methods have severe limitations regarding characterization of anisotropic compartments that are randomly oriented[3], a scenario that widely occurs in Nature and especially within grey matter. The angular d-PFG experiment[4,5] has been predicted to be able to detect compartment shape anisotropy[3] (csA) and microscopic anisotropy (μA) even when compartments are completely randomly oriented owing to its unique experimental parameters that can be varied (the angle ψ between the gradients and the mixing time (t_m)). Indeed, this was recently observed experimentally for the first time[6]. Thus, the angular d-PFG experiment offers a novel means to gain unique insight into the underlying microstructure of heterogeneous specimens.

Aims. To study highly heterogeneous specimens (ranging from phantoms to biological specimens to porous media) using angular d-PFG MR and compare the new insights into pore morphology available from angular d-PFG MR with the microstructural information available from conventional s-PFG methods.

Methods. Since some of the specimens in this study are characterized by extremely inhomogeneous magnetic fields that result in very large internal gradients, all experiments were performed using bipolar-s-PFG (bp-s-PFG) or bipolar-d-PFG (bp-d-PFG) experiments. All experiments were performed on a Bruker 8.4T magnet equipped with gradient coils capable of producing 195 G/cm in the x-, y- and z-directions. For a review on the angular d-PFG methodology, see Ref 7.

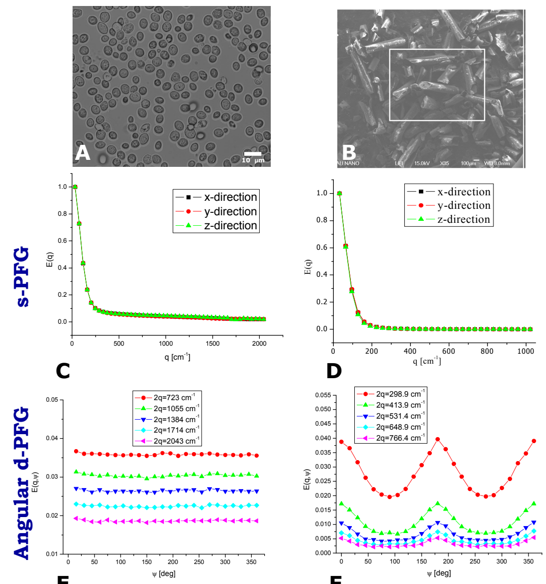

Figure 1. (A, B) Microscopy of yeast cells and randomly oriented cylinders (C, D) Bp-s-PFG experiments showing the lack of eA in both specimens. (E, F) Angular bp-d-PFG experiments at long t_m showing that yeast cells are spherical (no csA) while csA exists for the phantom, demonstrating that the compartments are randomly oriented anisotropic compartments.

Results and Discussion. To show that angular bp-d-PFG experiments can indeed offer novel microstructural information, we studied yeast cells, that are spherical (Fig 1A) and a phantom encompassing randomly oriented cylindrical compartments (Fig 1B). The s-PFG experiments in both specimens yielded an isotropic signal decay, as expected for these specimens, that do not have ensemble anisotropy[3] (Fig 1C and 1D). However, when we performed the angular bp-d-PFG experiments at long mixing times in both specimens, a dramatic difference was observed: the E(ψ) plots are flat for the spherical yeast cells (Fig 1E), while they are markedly modulated for randomly oriented cylinders (Fig 1F). This occurs since at long t_m, μA is completely decoupled from other effects, and the E(ψ) plots are only a manifestation of the compartment shape in the specimen[3]. The E(ψ) plots in spheres are flat since they are isotropic, thus they do not have csA, while the E(ψ) plots of randomly oriented cylinders show the pronounced modulation owing to their cylindrical shape. The same angular modulations were very recently observed in the grey matter, reporting on the presence of csA[8] and therefore on the random orientation of anisotropic compartments within the grey matter (it should be noted that the signal decay in s-PFG experiments was isotropic, offering no information on the underlying microstructure).

Another extremely important issue of diffusion MR is the diffusion modes of intra- and extracellular spaces. As a model system, we studied a Toluene-in-Water emulsion, where Toluene droplets are dispersed in water. In this system, the Toluene and H_2O peaks are well resolved on the chemical shift axis, and therefore can be probed separately. Figure 2A shows the bp-s-PFG experiments in such a system. The signal decay is isotropic, and surprisingly similar for both components, offering little microstructural information. However, when the angular bp-d-PFG experiments are performed with t_m=0 ms, bell-shaped functions emerge in the E(ψ) plots, reporting on microscopic anisotropy (i.e. on the presence of geometrical boundaries) for *both* components. In fact, the μA can be related to compartment size, and it seems that toluene is diffusing in a smaller compartment than water. To decouple the effects of μA, long t_m experiments[3] were performed. For toluene, the E(ψ) plots are almost completely flat, indicating that it is diffusing within an isotropic, spherical compartment; for H_2O however, the E(ψ) plots are strongly modulated, indicating that water molecules are confined to anisotropic spaces. In fact, the angular bp-d-PFG experiments offered nearly an image of the underlying microstructure, since they reported on the structural elements that are confining both emulsion components. Figure 2D shows the light microscopy image, validating that Toluene is diffusing in spheres (black) while water molecules undergo restricted, non-Gaussian diffusion in randomly oriented anisotropic compartments.

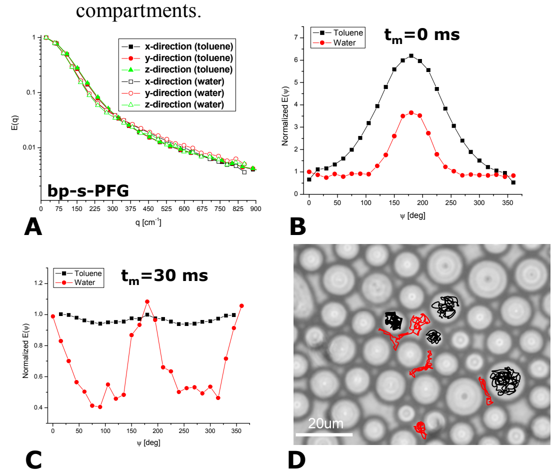

Figure 2. (A) bp-s-PFG experiments for both components of the emulsion. Note that the signal decay is isotropic for both. (B) Angular bp-d-PFG experiments with t_m=0 ms, reveals the presence of μA for both compartments. (C) Angular bp-d-PFG experiments with long t_m= reveals that toluene is diffusing within spheres, and the presence of csA for water, demonstrating that the water molecules are trapped in randomly oriented anisotropic compartments. (D) Light microscopy of the emulsion validates the angular bp-d-PFG results.

Conclusions. Angular bp-d-PFG is a powerful and promising new tool for studying diffusion and gaining novel noninvasive insights in microstructurally heterogeneous systems, even in the presence of large magnetic inhomogeneity. The angular bp-d-PFG is therefore extremely promising as a new source of contrast in d-PFG MRI of the CNS.

References. [1] Basser PJ et al., Biophys. J. 1994, 66:259. [2] Cohen Y and Assaf Y, NMR Biomed 2002, 15,516; Ong HH et al., Neuroimage 2008, 40:1619. [3] Özarslan E, J. Magn. Reson. 2009, 199:56. [4] Mitra PP, Phys. Rev. B, 1995, 51:15074. [5] Özarslan E, Basser PJ, J. Chem Phys. 2008, 128:154511. [6] Shemesh N et al., J. Chem. Phys. 2010, 133:044705. [7] Shemesh et al., NMR Biomed 2010, 23:757. [8] Shemesh N and Cohen Y, Magn. Reson. Med., 2010, Accepted for publication.