First experimental observation of both microscopic anisotropy (μA) and compartment shape anisotropy (CSA) in randomly oriented biological cells using double-PFG NMR

N. Shemesh, E. Özarslan, P. J. Basser, and Y. Cohen

1School of Chemistry, Tel Aviv University, Tel Aviv, Israel, 2Section on Tissue Biophysics and Biomimetics, NICHD, National Institutes of Health, Bethesda, MD, United States

Introduction. Single-pulsed-field-gradient (s-PFG) methods have been widely employed in diffusion NMR and MRI to characterize microstructures in the central-nervous-system. Notably, diffusion tensor imaging (DTI) provides a means to quantify the orientation of coherently packed anisotropic compartments such as white matter fascicles; the q-space approach offers a means to quantify the relative sizes of such structures. However, DTI does not provide microstructural information about anisotropic compartments that are randomly oriented; q-space approaches necessitate extremely strong gradients to characterize only relative sizes. These inherent limitations of s-PFG limit its usefulness in, inter-alia, grey matter structures which are randomly oriented. Moreover, these methods do not provide a signature for compartment shape. For example, randomly oriented ellipsoids or cylinders specimens produce the same qualitative signal decay in s-PFG, as do spherical compartments.

Double-PFG (d-PFG) is emerging as a new powerful tool for studying restricted diffusion, especially where s-PFG is inherently limited. The d-PFG, first proposed by Cory et al. in 1990 is an extension of s-PFG, and employs two gradient pairs \( G_1 \) and \( G_2 \), which are separated by a mixing time \( \tau_m \) (Fig. 1A). Another variant of d-PFG was recently introduced, in which the middle gradients are superimposed, so that \( \tau_m=0 \) ms, a desirable property for some applications (Fig 1B). The angular d-PFG experiment, in which the middle gradients are superimposed, reveals the size distribution of yeast cells from which the mean size was quantified. Moreover, the loss of angular dependence at longer \( \tau_m \) (Figure 2B, green symbols) implies that the yeast are spherical, which is consistent with the microscopy results. Figure 3 shows the experimental results from Fischerella cyanobacteria, which are locally anisotropic. Figure 3A shows the s-PFG, which yields an isotropic signal decay providing no information about the eccentricity of the cyanobacteria. However, inspecting the d-PFG angular experiment data at various \( \tau_m \) (Figure 3B) clearly shows that the signal is not flat, but that the characteristic curve of compartment shape anisotropy is obtained. Therefore one can infer that the cyanobacteria are not spherical. Similar results were obtained for both human and rat fixed mesenchymal stem cells (data not shown), demonstrating that the angular dependence can be obtained for fixed mammalian cells as well.

Conclusions. This study demonstrates that d-PFG provides novel microstructural information in randomly oriented biological cell systems. We demonstrated that the cell size can be measured to within the accuracy of 0.2 \( \mu \)m in yeast, and moreover, the mixing time dependence reveals microstructural information about compartment shape; such results can even be obtained on mammalian cells which do not have a cell wall. Future studies will focus on d-PFG imaging and its ability to provide new contrasts in grey matter and in pathological conditions.