Pulsed-field gradient NMR is an important tool to measure diffusion of proteins and protein assemblies and thus obtain insight into their structure and dynamics. For extended objects, such as amyloid fibrils, these experiments become difficult to interpret because in addition to translational diffusion they are also sensitive to rotational diffusion. We have constructed a mathematical theory describing the outcome of PFG NMR experiments on rod-like fibrils. These analytical results proved to be in excellent agreement with the predictions from our Monte-Carlo simulations. The effect of rotational diffusion is indeed significant. However, just like translational diffusion, rotational diffusion of a fibril is a slow process and registers as such in the PFG NMR experiments. Contrary to certain literature claims, this allows one to separate spectral signals from fibrils and other species that may be present in the sample (monomers, proteolytic fragments, etc.) based on their different diffusion properties. To test the validity of our theory, we have studied fibrils formed by protein Sup35NM derived from yeast translation termination factor Sup35p. The presence of disordered M domain in Sup35NM fibrils makes it possible to observe spectral signals from the said fibrils. Using this system, we have shown that the signals originating from the flexible tail of the peptide chains comprising the body of the fibril can be successfully separated from the similar signals representing monomers or proteolytic fragments of Sup35NM. This research was supported by RSF grant 15-14-20038.

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