Tracing Molecular Propagation in Dextran Solutions by Pulsed Field Gradient NMR

ARTICLE *in* JOURNAL OF PHYSICAL CHEMISTRY LETTERS · JUNE 2012

Impact Factor: 7.46 · DOI: 10.1021/jz300734m

3 AUTHORS, INCLUDING:

Alexander Shakhov
University of Leipzig
4 PUBLICATIONS **17** CITATIONS
SEE PROFILE

Rustem Valiullin
University of Leipzig
137 PUBLICATIONS **1,673** CITATIONS
SEE PROFILE

THE JOURNAL OF PHYSICAL CHEMISTRY Letters

Letter

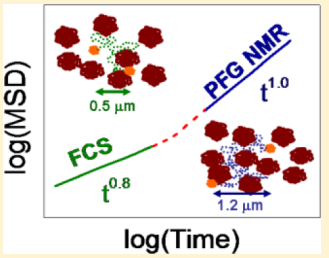
pubs.acs.org/JPCL

Tracing Molecular Propagation in Dextran Solutions by Pulsed Field Gradient NMR

Alexander Shakhov, Rustem Valiullin,* and Jörg Kärger

Faculty of Physics and Earth Sciences, University of Leipzig, Linnéstrasse 5, D-04103 Leipzig, Germany

ABSTRACT: We have exploited the pulsed field gradient (PFG) technique of NMR to measure molecular diffusion in aqueous solutions of a mixture of dextran molecules. From detailed studies by fluorescence correlation spectroscopy (FCS), the lighter component of such mixtures is known to undergo subdiffusion, up to diffusion path lengths on the order of 0.5 μm. Our studies provide clear evidence of a crossover to normal diffusion for diffusion path lengths from this range up to about 1 μm.



SECTION: Kinetics and Dynamics

Molecular transport in biological systems plays a decisive role in functioning of living organisms.[1] In biological media, the rates of molecular rearrangement and, therefore, of biochemical reactions are strongly affected by molecular crowding and confinement.[2] In particular, there is much experimental evidence that translational dynamics of various cell constitutes may undergo subdiffusion.[3−14] Here, in contrast to the process of normal diffusion, the mean square displacement (MSD) in a given direction during their erratic excursions in highly inhomogeneous environments increases less than linearly with the observation time

$$\langle x^2(t)\rangle \propto t^{\alpha} \qquad \alpha < 1. \tag{1}$$

In normal diffusion, most often obtained in physical systems under equilibrium conditions, the time exponent α is 1, yielding the well-known Einstein relation

$$\langle x^2(t)\rangle = 2Dt \tag{2}$$

with the proportionality factor D referred to as self-diffusivity. The deviations observed from eq 2 are referred to as the existence of substructures formed in the process of "molecular crowding", which appear in a wealth of structural heterogeneities stipulated by the differences in the sizes of the constituents. It has to be noted, however, that the subdiffusive behavior under such conditions is not a universal phenomenon, and there are many examples of normal diffusion in highly crowded media.[15] Therefore, an experimental clarification of the intrinsic mechanisms giving rise to the anomalies in Brownian motion in systems, in which they are observed, using more advanced experimental techniques or combinations of several techniques covering different length and time scales, is of immediate importance for their classification and further possible generalization.

Most importantly, for the application of complementary experimental techniques for studying diffusion in crowded media, anomalous diffusion has been found to occur already in systems containing not more than two differently sized macromolecules, which may be considered as the most simple variant imaginable for mimicking such systems. Application of fluorescence correlation spectroscopy (FCS)[16,17] to aqueous solutions of two differently sized dextrans, with well-established procedures of data analysis, provides clear evidence that the smaller one of the dextrans undergoes subdiffusion.[5,6] By combining the results of FCS with simulations, in ref 9, the properties of crowding-induced subdiffusion are shown to be consistent with the predictions for fractional Brownian motion or obstructed (percolation-like) diffusion and to notably deviate from the pattern to be expected for continuous-time random walk, which is known to yield differences between the time and ensemble averages of the MSD.[11,18−20] Further, the subdiffusive patterns observed have been suggested to result from anticorrelations in the course of Brownian motion having viscoelastic origin.[21,22]

Though, as a consequence of substructure formation, subdiffusion under molecular crowding is observable over distances exceeding the size of the involved molecules by more than 2 orders of magnitude,[5] with further increasing diffusion path lengths, the central limit theorem of statistics suggests final transition to normal diffusion. This has to be expected as soon as the displacements notably exceed the correlation lengths of structural heterogeneity. In this case, the total diffusion path length may be understood as the superposition of displacements in subsequent intervals of time with identical probability distributions, giving rise to a Gaussian with a mean square width increasing linearly with time. Transition between subdiffusion and normal diffusion may be taken, therefore, as the measure of the correlation lengths of the substructures formed by molecular crowding.

Received: June 6, 2012
Accepted: June 26, 2012

The present Letter is dedicated to this very issue and reports about diffusion studies with aqueous solutions of dextran using the pulsed field gradient (PFG) technique of NMR. It is worth noting that similar diffusion investigations have already been performed. However, they have mostly been concerned with molecular-mass and concentration dependencies of guest molecules in dextran solutions.[23−25] The diffusive transport at different length scales has remained unexplored. From initially more than 10 μm,[26] today, owing to the use of PFGs, NMR diffusion studies can be performed over diffusion path lengths of typically micrometers, attaining, under favorable conditions, even values below 100 nm.[27−30] With this option, magnetic field gradient NMR techniques have been proven to be an effective tool for the exploration of anomalous and restricted diffusion in various systems.[31−37]

In our work, we used an NMR spectrometer operating at 400 MHz proton resonance frequency, equipped with a home-built PFG unit, allowing the application of magnetic field gradients with amplitudes up to 35 T/m with very short rise and fall times.[30] By generating the NMR signal with the stimulated-echo pulse sequence,[38,39] observation times from t = 30 up to 800 ms have been considered. The measurements have been performed with 40 and 640 kDa dextrans (Sigma-Aldrich, Germany) dissolved in deuterated water, both as single-component solutions of 5 wt % and in a 75% 640 kDa to 25% 40 kDa mixture in an aqueous solution of 20 wt %. Among the various dextran mixture compositions considered in refs 5 and 6, the latter composition was selected as a compromise for attaining sufficiently large transverse nuclear magnetic relaxation times, necessary for enabling measurements over the considered time and space scales (facilitated by the use of light molecules at high dilutions), under conditions where, with time exponents of $\alpha \approx 0.8$, FCS unmistakably points to subdiffusion, the occurrence of which, vice versa, is promoted by increasing molecular sizes and concentrations.

Figure 1 shows the PFG NMR signal attenuation curves as the primary data of the PFG NMR diffusion studies for the pure components. For a single-component system undergoing normal diffusion with a diffusivity D, the PFG NMR attenuation curve is known to follow a simple exponential of the form[38,39]

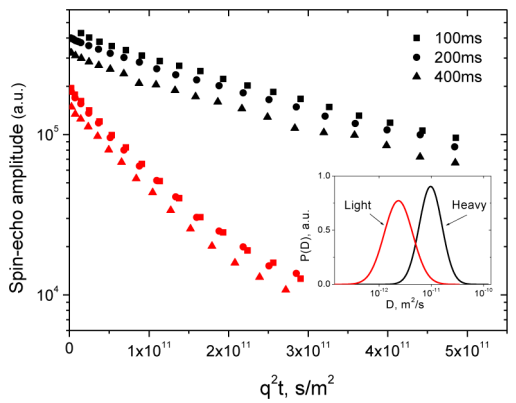


Figure 1. Spin−echo diffusion attenuation functions for heavy and light dextran aqueous solutions measured at different observation times indicated in the figure. The inset shows the normalized diffusivity distribution functions $p(D)$ obtained by fitting eq 6, combined with eqs 3 and 7, to the experimental data.

$$\Psi_s = \Psi_0 \exp\{-q^2 D t\} \tag{3}$$

with q representing a measure of the strength of the magnetic field gradient pulses applied. Equation 3 may as well be applied for systems deviating from normal diffusion. In this case, the parameter D appearing in this equation becomes an "effective" diffusivity, defined by the relation

$$D_{\text{eff}} = \langle x^2(t)\rangle/2t \tag{4}$$

as the ratio between the MSD in the direction of the applied field gradient and (two times) the observation time. For normal diffusion, with eq 2, the thus defined parameter is clearly seen to coincide with the self-diffusivity. For subdiffusion, however, with eqs 1 and 4, the diffusivities recorded in PFG NMR measurements are seen to follow the relation

$$D_{\text{eff}} \propto t^{-(1-\alpha)} \tag{5}$$

that is, to decrease with increasing time, rather than being constant.

The attenuation curves shown in Figure 1 are clearly seen to not comply with the simple pattern predicted by eq 3. We have to take into account, however, that, as a consequence of mass distribution inherent to each of the dextran samples, there is also a distribution $p(D)$ in the diffusivities of each component.[40,41] Under such conditions, the PFG NMR signal attenuation results as a superposition

$$\Psi = \int p(D)\Psi_s(D)\, dD \tag{6}$$

rather than as a single exponential. Although $p(D)$ is generally not known, it could often be well-captured by the log-normal distribution[42]

$$p(D) = (\sqrt{2\pi}Dw)^{-1} \exp\left\{-\frac{(\ln D - \ln D_0)^2}{2w^2}\right\} \tag{7}$$

where D_0 is the average diffusivity and w is the distribution width. The inset in Figure 1 shows the diffusivity distribution functions for the two single-component 5% aqueous solutions of dextrans obtained by fitting eq 6 to the experimental data. Here, $p(D)$ has been used as given by eq 7 and $\Psi_s(D)$ as given by eq 3. It has to be mentioned that Ψ_0, which takes account of the transverse and longitudinal rates of nuclear magnetic relaxation of the polymer molecules, has been taken to be identical for each mass fraction. In our case, due to the high dilution of the dextran molecules and the relatively narrow mass distributions, this is well-justified.[43] Notably, the distributions $p_{\text{light}}(D)$ and $p_{\text{heavy}}(D)$ resulting for the two different dextran samples with the molecular masses of 40 and 640 kDa, respectively, do not overlap heavily and can thus be easily separated from each other in the PFG NMR diffusion measurements with the two components in a mixture.

Figure 2 shows the PFG NMR attenuation curves for the two-component system for the total range of diffusion times 30 $\leq t \leq$ 800 ms studied in the experiments. Indicated by the full line is the attenuation curve obtained via eq 7 with a distribution function $p(D)$ as resulting from the weighted superposition of the distribution functions of the two constituents shown in Figure 1, namely

$$\Psi = \sum_{i=1,2} \int f_i p_i(D)\Psi_i(D)\, dD \tag{8}$$

dx.doi.org/10.1021/jz300734m | J. Phys. Chem. Lett. 2012, 3, 1854−1857

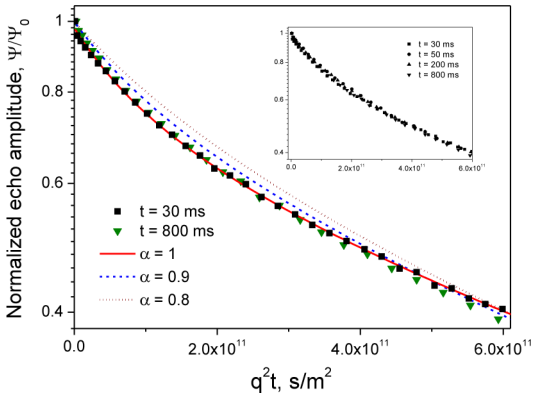


Figure 2. Normalized spin−echo diffusion attenuation functions for the 20% aqueous solution of dextran mixture (75 wt % of 640 kDa dextran and 25 wt % of 40 kDa dextran) for two diffusion times, 30 and 800 ms. The solid line shows the best fit of eq 8 to the experimental data. The broken lines show the attenuation curves resulting from the analytical expressions of eqs 8, 6, and 5 for diffusion time t = 800 ms, assuming subdiffusion with the exponents α = 0.8 (dots) and 0.9 (dashes) for the lighter dextran component. The inset shows the same graph, including the experimental data for the intermediate observation times.

with the subscript i referring to the two dextran components (light and heavy), f_i denoting the proton fractions (in our case, coinciding with the weight fractions) of the two components, $p_i(D)$ as given by eq 7 with the distribution widths w obtained from the single-component solutions, and $\Psi_i(D)$ as given by eq 3. In this way, we have assumed that, upon mixing, only the mean diffusivities $D_{0,i}$ of the mixture components do change, while their distributions, as compared to the single-component dextrans, remain invariant. It is shown by the solid line in Figure 2 that in this way, with only two fitting parameters $D_{0,i}$, an excellent fit of eq 8 to the experimental data is obtained.

As observed already with the single-component systems in Figure 1, the PFG NMR signal attenuation is found to be invariant with time. This result was indeed expected for the single-component systems, which are implied to undergo normal diffusion. However, also the mixture data displayed in Figure 2 are now found to yield, within the limits of accuracy, no indication of a time dependence. For all observation times ($30 \le t \le 800$ ms), the best fit of the experimental attenuation data was attained with time-invariant diffusivities, namely, with values of $D_{light} = (8.7 \pm 1.0) \times 10^{-12}$ m^2 s^{-1} and $D_{heavy} = (9.70 \pm 0.35) \times 10^{-13}$ m^2 s^{-1}, respectively, for the two dextran components.

To assess the significance of this finding, we have to check how strong the deviation of the time exponent α, from α = 1 for normal diffusion, has to be for giving rise to a clearly visible splitting in the $\ln \Psi$ versus q^2t attenuation plots for different observation times. This is illustrated in Figure 2 by the dashed and dotted lines. They show the attenuation plots, which, on the basis of the data for t = 30 ms, must be expected for an observation time of t = 800 ms if the lighter dextran component would undergo subdiffusion with time exponents α = 0.9 and 0.8, respectively. With eq 5, in this case, the diffusivity for t = 800 ms would amount to

$$D_{light}(t = 800\ \text{ms}) = 8.7 \times 10^{-12}\ \text{m}^2\text{s}^{-1}\left(\frac{30\ \text{ms}}{800\ \text{ms}}\right)^{1-\alpha} \quad (9)$$

rather than to $D_{light} = 8.7 \times 10^{-12}$ m^2 s^{-1}, which holds for t = 30 ms.

As to be expected from eq 5, the thus calculated PFG NMR attenuation plots for t = 30 ms exhibit, in their first fast decay corresponding to the lighter component, a decrease in decay with increasing observation time, corresponding to the decreasing diffusivity. Noting that the experimental error of our measurements in Figure 2 does not exceed the symbol size, comparison between the experimental data and the calculations clearly indicates that, over the considered space and time scales, subdiffusion with time exponent α = 0.8 can be ruled out, while a value of $\alpha \approx 0.9$ can be considered as a reasonable lower limit.

With eq 2, the minimum diffusion path lengths of the light dextran molecules in our experiments are found to be of about 1.2 μm. Having in mind that the maximum path lengths covered in FCS, being on the order of 0.5 μm, provide clear evidence of subdiffusion with $\alpha \approx 0.8$, with the present findings, the transition from sub- to normal diffusion can be estimated to occur in exactly this intermediate range, that is, from about 0.5 to 1.2 μm.

Within the present studies, the potentials of PFG NMR for tracing short displacements could only partially be exploited. Some of the present limitations may be overcome by the use of larger dextrans, simultaneously with the application of perdeuterated species for the larger component so that the total NMR signal may be expected to stem, just as in FCS, from only those molecules that are in the focus of the diffusion studies and which, clearly, notably enhance the accuracy of the measurements. Such experiments are in preparation. Following first experiments with nanoporous host−guest systems,[44] the benefit of such studies will be further enhanced if, by application to one and the same system, it becomes possible to directly correlate the messages on ensemble diffusion (with typically 10^{18} spins) as attainable by PFG NMR with those on the diffusion of small-number entities as attainable with FCS[17] and single-particle tracking[45,46] and to explore, in this way, by direct experimental evidence, the occurrence (or breaking) of ergodicity[11,14,18−20] under the conditions of molecular crowding.

AUTHOR INFORMATION

Corresponding Author

*E-mail: valiullin@uni-leipzig.de.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We are obliged to Matthias Weiss, Bayreuth, for stimulating discussions and to the German Science Foundation (Research Unit 877 “From Local Constraints to Macroscopic Transport”) for financial support.

REFERENCES

(1) Verkman, A. S. Solute and Macromolecule Diffusion in Cellular Aqueous Compartments. *Trends Biochem. Sci.* **2002**, *27*, 27−33.

(2) Minton, A. P. The Influence of Macromolecular Crowding and Macromolecular Confinement on Biochemical Reactions in Physiological Media. *J. Biol. Chem.* **2001**, *276*, 10577−10580.

(3) Feder, T. J.; Brust-Mascher, I.; Slattery, J. P.; Baird, B.; Webb, W. W. Constrained Diffusion or Immobile Fraction on Cell Surfaces: A New Interpretation. *Biophys. J.* **1996**, *70*, 2767−2773.

(4) Saxton, M. J.; Jacobson, K. Single-Particle Tracking: Applications to Membrane Dynamics. *Annu. Rev. Biophys. Biomol. Struct.* **1997**, *26*, 373−399.
(5) Weiss, M.; Elsner, M.; Kartberg, F.; Nilsson, T. Anomalous Subdiffusion Is a Measure for Cytoplasmic Crowding in Living Cells. *Biophys. J.* **2004**, *87*, 3518−3524.
(6) Banks, D. S.; Fradin, C. Anomalous Diffusion of Proteins Due to Molecular Crowding. *Biophys. J.* **2005**, *89*, 2960−2971.
(7) Ritchie, K.; Shan, X. Y.; Kondo, J.; Iwasawa, K.; Fujiwara, T.; Kusumi, A. Detection of Non-Brownian Diffusion in the Cell Membrane in Single Molecule Tracking. *Biophys. J.* **2005**, *88*, 2266−2277.
(8) Lubelski, A.; Klafter, J. Temporal Correlation Functions of Concentration Fluctuations: An Anomalous Case. *J. Phys. Chem. B* **2008**, *112*, 12740−12747.
(9) Szymanski, J.; Weiss, M. Elucidating the Origin of Anomalous Diffusion in Crowded Fluids. *Phys. Rev. Lett.* **2009**, 103.
(10) Wu, J.; Berland, K. M. Propagators and Time-Dependent Diffusion Coefficients for Anomalous Diffusion. *Biophys. J.* **2008**, *95*, 2049−2052.
(11) He, Y.; Burov, S.; Metzler, R.; Barkai, E. Random Time-Scale Invariant Diffusion and Transport Coefficients. *Phys. Rev. Lett.* **2008**, 101.
(12) Golding, I.; Cox, E. C. Physical Nature of Bacterial Cytoplasm. *Phys. Rev. Lett.* **2006**, *96*, 098102−098104.
(13) Fakhri, N.; MacKintosh, F. C.; Lounis, B.; Cognet, L.; Pasquali, M. Brownian Motion of Stiff Filaments in a Crowded Environment. *Science* **2010**, *330*, 1804−1807.
(14) Jeon, J.-H.; Tejedor, V.; Burov, S.; Barkai, E.; Selhuber-Unkel, C.; Berg-Sørensen, K.; Oddershede, L.; Metzler, R. In Vivo Anomalous Diffusion and Weak Ergodicity Breaking of Lipid Granules. *Phys. Rev. Lett.* **2011**, *106*, 048103.
(15) Dix, J. A.; Verkman, A. S., Crowding Effects on Diffusion in Solutions and Cells. In *Annual Review of Biophysics*; Annual Reviews: Palo Alto, CA, 2008; Vol. *37*, pp 247−263.
(16) Saxton, M. J. Anomalous Subdiffusion in Fluorescence Photobleaching Recovery: A Monte Carlo Study. *Biophys. J.* **2001**, *81*, 2226−2240.
(17) Ries, J.; Schwille, P. Fluorescence Correlation Spectroscopy. *BioEssays* **2012**, *34*, 361−368.
(18) Magdziarz, M.; Weron, A.; Burnecki, K.; Klafter, J. Fractional Brownian Motion Versus the Continuous-Time Random Walk: A Simple Test for Subdiffusive Dynamics. *Phys. Rev. Lett.* **2009**, *103*, 180602−180604.
(19) Weber, S. C.; Spakowitz, A. J.; Theriot, J. A. Bacterial Chromosomal Loci Move Subdiffusively through a Viscoelastic Cytoplasm. *Phys. Rev. Lett.* **2010**, 104.
(20) Lubelski, A.; Sokolov, I. M.; Klafter, J. Nonergodicity Mimics Inhomogeneity in Single Particle Tracking. *Phys. Rev. Lett.* **2008**, 100.
(21) Ernst, D.; Hellmann, M.; Kohler, J.; Weiss, M. Fractional Brownian Motion in Crowded Fluids. *Soft Matter* **2012**, *8*, 4886−4889.
(22) Hellmann, M.; Klafter, J.; Heermann, D. W.; Weiss, M. Challenges in Determining Anomalous Diffusion in Crowded Fluids. *J. Phys.: Condens. Matter* **2011**, 23.
(23) Furukawa, R.; Arauzlara, J. L.; Ware, B. R. Self-Diffusion and Probe Diffusion in Dilute and Semidilute Aqueous-Solutions of Dextran. *Macromolecules* **1991**, *24*, 599−605.
(24) Brown, W.; Stilbs, P. Self-Diffusion of Poly(Ethylene Oxide) in Aqueous Dextran Solutions Measured Using FT-Pulsed Field Gradient NMR. *Polymer* **1983**, *24*, 188−192.
(25) Watanabe, T.; Ohtsuka, A.; Murase, N.; Barth, P.; Gersonde, K. NMR Studies on Water and Polymer Diffusion in Dextran Gels. Influence of Potassium Ions on Microstructure Formation and Gelation Mechanism. *Magn. Reson. Med.* **1996**, *35*, 697−705.
(26) Woessner, D. E. N.M.R. Spin-Echo Self-Diffusion Measurements of Fluids Undergoing Restricted Diffusion. *J. Phys. Chem.* **1963**, *67*, 1365−1367.
(27) Kimmich, R.; Unrath, W.; Schnur, G.; Rommel, E. NMR Measurement of Small Self-Diffusion Coefficients in the Fringe-Field of Superconducting Magnets. *J. Magn. Reson.* **1991**, *91*, 136−140.
(28) Valiullin, R.; Skirda, V. D.; Stapf, S.; Kimmich, R. Molecular Exchange Processes in Partially Filled Porous Glass as Seen with NMR Diffusometry. *Phys. Rev. E* **1997**, *55*, 2664−2671.
(29) Callaghan, P. T.; Komlosh, M. E.; Nyden, M. High Magnetic Field Gradient PGSE NMR in the Presence of a Large Polarizing Field. *J. Magn. Reson.* **1998**, *133*, 177−182.
(30) Galvosas, P.; Stallmach, F.; Seiffert, G.; Kärger, J.; Kaess, U.; Majer, G. Generation and Application of Ultra-High-Intensity Magnetic Field Gradient Pulses for NMR Spectroscopy. *J. Magn. Reson.* **2001**, *151*, 260−268.
(31) Appel, M.; Fleischer, G.; Kärger, J.; Fujara, F.; Siegel, S. NMR Evidence of Anomalous Molecular Diffusion Due to Structural Confinement. *Europhys. Lett.* **1996**, *34*, 483.
(32) Kimmich, R.; Fatkullin, N. Polymer Chain Dynamics and NMR. In *NMR/3D Analysis/ Photopolymerization*; Springer Verlag: Berlin, Germany, 2004; Vol. *170*, pp 1−113.
(33) Latour, L. L.; Mitra, P. P.; Kleinberg, R. L.; Sotak, C. H. Time-Dependent Diffusion-Coefficient of Fluids in Porous-Media as a Probe of Surface-to-Volume Ratio. *J. Magn. Reson. A* **1993**, *101*, 342−346.
(34) Kopf, M.; Corinth, C.; Haferkamp, O.; Nonnenmacher, T. F. Anomalous Diffusion of Water in Biological Tissues. *Biophys. J.* **1996**, *70*, 2950−2958.
(35) Zakhartchenko, N. L.; Skirda, V. D.; Valiullin, R. R. Self-Diffusion of Water and Oil in Peanuts Investigated by PFG NMR. *Magn. Reson. Imaging* **1998**, *16*, 583−586.
(36) Pfeuffer, J.; Flogel, U.; Dreher, W.; Leibfritz, D. Restricted Diffusion and Exchange of Intracellular Water: Theoretical Modelling and Diffusion Time Dependence of ^{1}H NMR Measurements on Perfused Glial Cells. *NMR Biomed.* **1998**, *11*, 19−31.
(37) Ozarslan, E.; Basser, P. J.; Shepherd, T. M.; Thelwall, P. E.; Vemuri, B. C.; Blackband, S. J. Observation of Anomalous Diffusion in Excised Tissue by Characterizing the Diffusion-Time Dependence of the MR Signal. *J. Magn. Reson.* **2006**, *183*, 315−323.
(38) Callaghan, P. T. *Principles of Nuclear Magnetic Resonance Microscopy*; Clarendon Press: Oxford, U.K., 1991.
(39) Price, W. S. *NMR Studies of Translational Motion*; University Press: Cambridge, U.K., 2009.
(40) Hakansson, B.; Nyden, M.; Soderman, O. The Influence of Polymer Molecular-Weight Distributions on Pulsed Field Gradient Nuclear Magnetic Resonance Self-Diffusion Experiments. *Colloid Polym. Sci.* **2000**, *278*, 399−405.
(41) Miller, M. C.; Klyosov, A.; Platt, D.; Mayo, K. H. Using Pulse Field Gradient NMR Diffusion Measurements to Define Molecular Size Distributions in Glycan Preparations. *Carbohydr. Res.* **2009**, *344*, 1205−1212.
(42) Walderhaug, H.; Soderman, O.; Topgaard, D. Self-Diffusion in Polymer Systems Studied by Magnetic Field-Gradient Spin-Echo NMR Methods. *Prog. Nucl. Magn. Reson. Spectrosc.* **2010**, *56*, 406−425.
(43) Maklakov, A. I.; Skirda, V. D.; Fatkullin, N. F. Self-Diffusion in Polymer Systems. In *Encyclopedia of Fluid Mechanics*; Chremisinoff, N. M., Ed.; Gulf-Publishing Co.: Houston, TX, 1990; Vol. *9*.
(44) Feil, F.; Naumov, S.; Michaelis, J.; Valiullin, R.; Enke, D.; Kärger, J.; Bräuchle, C. Single-Particle and Ensemble Diffusivities—Test of Ergodicity. *Angew. Chem.* **2012**, *124*, 1178−1181.
(45) Zürner, A.; Kirstein, J.; Doblinger, M.; Bräuchle, C.; Bein, T. Visualizing Single-Molecule Diffusion in Mesoporous Materials. *Nature* **2007**, *450*, 705−708.
(46) Bräuchle, C.; Lamb, D. C.; Michaelis, J. *Single Particle Tracking and Single Molecule Energy Transfer*; Wiley-VCH: Weinheim, Germany, 2010.