In vivo observation and biophysical interpretation of time-dependent diffusion in human cortical gray matter

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Abstract

The dependence of the diffusion MRI signal on the diffusion time \( t \) is a hallmark of tissue microstructure at the scale of the diffusion length. Here we measure the time-dependence of the mean diffusivity \( D(t) \) and mean kurtosis \( K(t) \) in cortical gray matter and in 25 gray matter sub-regions, in 10 healthy subjects. Significant diffusivity and kurtosis time-dependence is observed for \( t > 21.2-100 \) ms, and is characterized by a power-law tail \( \sim t^{-\vartheta} \) with dynamical exponent \( \vartheta \). To interpret our measurements, we systematize the relevant scenarios and mechanisms for diffusion time-dependence in the brain. Using effective medium theory formalisms, we derive an exact relation between the power-law tails in \( \sim t^{-\vartheta} \) and is characterized by a power-law tail \( \vartheta \) dependent dMRI signal \( \vartheta \) used in clinical studies. Furthermore, measurement of the time-scale of the diffusion length \( \vartheta \) determined from the time-dependence of the dMRI signal in the presence of randomly positioned restrictions along neurites. We analyze the short-range disordered statistics of synapses on axon collaterals in the cortex, and perform one-dimensional Monte Carlo simulations of diffusion restricted by permeable barriers with a similar randomness in their placement, to confirm the \( \vartheta = 1/2 \) exponent. In contrast, the Kärger model of exchange is less consistent with the data since it does not capture the diffusivity time-dependence, and the estimated exchange time from \( K(t) \) falls below our measured \( t \)-range. Although we cannot exclude exchange as a contributing factor, we argue that structural disorder along neurites is mainly responsible for the observed time-dependence of diffusivity and kurtosis. Our observation and theoretical interpretation of the \( t^{-1/2} \) tail in \( D(t) \) and \( K(t) \) altogether establish the sensitivity of a macroscopic MRI signal to micrometer-scale structural heterogeneities along neurites in human gray matter in vivo.

1. Introduction

The effect of varying the diffusion time \( t \) on the diffusion MRI (dMRI) signal has been studied in neuronal tissue since the 1990’s (Horsfield et al., 1994; Beaulieu and Allen, 1996; Stanisz et al., 1997; Assaf and Cohen, 2000; Does et al., 2003), and has been increasingly used for quantifying neuronal microstructure (Nilsson et al., 2009; Kunz et al., 2013; Pyatigorskaya et al., 2014; Novikov et al., 2014; Wu et al., 2014; Burcaw et al., 2015; Fieremans et al., 2016; Palombo et al., 2016; Jespersen et al., 2018; Lee et al., 2018), as reviewed by Novikov et al. (2019). Such investigations are of interest, as they are complementary to traditional \( q \)-space imaging at fixed \( t \), widely used in clinical studies. Furthermore, measurement of the time-dependent dMRI signal offers a direct probe to restrictions at the scale of the diffusion length \( L(t) = \sqrt{\langle x^2(t) \rangle} \sim 1-10 \) \( \mu \)m (defined as root-mean-squared molecular displacement), and in principle allows one to classify (Novikov et al., 2014) and quantify the corresponding microstructural features in brain non-invasively (Latour et al., 1994; Barazany et al., 2009; Burcaw et al., 2015; Fieremans et al., 2016; De Santis et al., 2016; Benjamin et al., 2016; Lee et al., 2018).

So far, the time-dependence of the dMRI signal has been most often studied by measuring the diffusion coefficient \( D(t) = \langle x^2 \rangle/2t \). In gray matter, frequency dependence of the diffusion coefficient, \( D(\omega) \), was previously reported in rat cortical areas using oscillating gradient spin echo techniques (OGSE) between 20–1000 Hz (Does et al., 2003), and in mouse brain between 50-150 Hz (Aggarwal et al., 2012). Similiar OGSE techniques revealed \( D(\omega) \) in adult mouse cerebellum (Wu et al., 2014). In addition, the diffusion kurtosis, \( K(t) = \langle x^4 \rangle/\langle x^2 \rangle^2 - 3 \) (Kiselev, 2010; Jensen and Helpern, 2010), was shown to have a non-monotonic behavior at short times in rat cortex, between 2 and 29 ms, using both conventional PGSE (pulsed gradient spin echo) and OGSE techniques (Pyatigorskaya et al., 2014). The same PGSE and OGSE techniques were used to study \( D(t) \) and \( K(t) \) in healthy and injured mouse brains (Wu et al., 2018). Using numerical simulations, the finer microstructure of dendrites has been studied by constructing artificial spines along dendrites and investigating the time-dependence of an intra-dendritic diffusion coefficient (Palombo et al., 2017). However, the time-dependence of the dMRI signal in cortical areas of the human brain in vivo has not yet been investigated.

Here we measure \( D(t) \) and \( K(t) \) in vivo in human cortical gray matter for \( t = 21.2-100 \) ms using a standard clinical PGSE sequence at fixed echo time on a clinical scanner. To interpret our measurements, we consider the effect of coarse graining of the structural disorder by diffusion, and the effect of water exchange, on \( D(t) \) and \( K(t) \).

Structural disorder causes the time-dependence of diffusion metrics (Novikov et al., 2014). With increasing diffusion time \( t \),
water molecules coarse-grain the underlying micro-architecture over increasing length scales $L(t)$, such that, qualitatively, a medium (e.g., a tissue compartment) can be effectively viewed as a set of domains of the size $\sim L(t)$, each with a different local diffusion coefficient $D(x_0)L(t)$ (Novikov et al., 2019). While averaging over $L \to \infty$ would completely homogenize the medium, resulting in asymptotically Gaussian diffusion with effective $D_{L=\infty} \equiv D_{G}$, at finite $t$ and $L(t)$, such coarse-graining is incomplete. This gradual approach to Gaussian diffusion manifests itself in a characteristic inverse power-law time-dependence of $D(t)$ within a given tissue compartment (Novikov et al., 2014; Burcaw et al., 2015; Fieremans et al., 2016; Jespersen et al., 2018). Likewise, the higher-order cumulants, such as $K(t)$, acquire time-dependence (Novikov and Kiselev, 2010; Burcaw et al., 2015; Dhital et al., 2017) due to incomplete coarse-graining (as a measure of the residual inhomogeneity of the effective medium). The same underlying physics of coarse-graining results in the power-law behavior $t^{-\alpha}$ of both $D(t)$ and $K(t)$ at long diffusion times with identical power-law exponents (Burcaw et al., 2015; Dhital et al., 2017).

A competing mechanism for time-dependent kurtosis, $K(t)$, may be the exchange between compartments — relevant even when diffusion in each of them can be already considered Gaussian at a given diffusion time (Fieremans et al., 2010). The way to think about the diffusion physics in this situation is to imagine that coarse-graining has already completed in each compartment, with slow exchange remaining between compartments. In this case, the overall $D(t)$ remains time-independent (as a weighted average of Gaussian compartment diffusivities), while the kurtosis decreases to zero asymptotically as $1/t$ as a manifestation of exchange.

As these two mechanisms result in distinct time-dependencies, studying both $D(t)$ and $K(t)$ with a focus on their asymptotic behavior at long $t$ offers ways to probe the relevant microstructural degrees of freedom — e.g., the presence of intra-compartmental non-Gaussianity connected to incomplete coarse-graining, and the related disorder universality class and/or effective dimensionality, as well as the importance of exchange between compartments, and the relative role of intra- and inter-compartmental kurtosis.

The outline of this work is as follows. In Section 2, we put the relevant mechanisms, such as the disorder coarse-graining picture, and the exchange picture, into overarching context of a model-selection tree for the brain microstructure, Fig. 1. We then present our experimental setup (Section 3) and results (Section 4). To interpret our experimental findings, we explore relevant branches of the model-selection tree, by deriving exact relations, Eqs. (11) and (12), between power-law tails in $D(t)$ and $K(t)$ (Section 2 and Appendix A), and by performing Monte Carlo simulations of one-dimensional diffusion in the presence of short-range disorder, with restrictions mimicking those along synapses on axon collaterals in the cortex based on our analysis of microscopy data (Section 4 and Appendix B). We discuss our theoretical and experimental results in Section 5.

### 2. Theory

In this Section we introduce the hierarchy of models that describe the connection between $D(t)$ and $K(t)$ with the various compartments and microstructure types that are likely to be present in the brain (Fig. 1). The resulting “selection tree” summarizes the various diffusion models from top to bottom, such as Gaussian diffusion in exchanging compartments, or diffusion in the presence of microstructure. Moving down the tree’s nodes is decided based on the presence or absence of time-dependence of $D$ and/or $K$. The selection tree illustrates that measuring the fourth-order cumulant (kurtosis) is essential to reveal the physical picture of the system of interest. Note that subsections in this Section are numbered based on the selection tree nodes in Fig. 1.

**Node 1: Gaussian compartments**

This is a broad class of phenomena where coarse-graining over the microstructure in each compartment has already happened, so that for all practical purposes, all compartments can be considered as homogeneous at the scale of $L(t)$ probed by the measurement. In this case, neither compartment diffusivity depends on time, and therefore, the overall $D = \text{const.}$

**Node 1.2: Single Gaussian compartment**

The simplest case is molecular diffusion in a uniform medium, i.e. a Gaussian compartment. This results in no time-dependence in the diffusion coefficient and zero kurtosis (as well as all higher-order cumulants); examples are pure liquids.

**Node 1.1.1: Non-exchanging Gaussian compartments**

A non-zero kurtosis indicates the presence of multiple compartments (which can be anisotropic). This physical picture underpins, e.g., the so-called Standard Model of diffusion in white matter (Novikov et al., 2019), generalizing a number of previous works (Kroenke et al., 2004; Jespersen et al., 2007, 2010; Fieremans et al., 2011; Zhang et al., 2012; Sotiropoulos et al., 2012; Jensen et al., 2016; Reisert et al., 2017; Novikov et al., 2018), some of which have been also applied to gray matter. In this case, one compartment consists of so-called “sticks” (Kroenke et al., 2004; Jespersen et al., 2007), i.e., narrow impermeable cylinders of finite diffusivity in the direction of the principal axis and negligible transverse diffusivity — modeling neurites. Other compartments then include the extra-neurite space as a locally Gaussian compartment, and possibly CSF as yet another distinct Gaussian compartment. In all such model variations, the diffusion coefficient and kurtosis (tensors) are time-independent. In the general case of $n$ non-interacting Gaussian compartments with fractions $p_i$ and (directional) diffusivities $D_i$, the overall diffusivity

$$\bar{D} = \sum_{i=1}^{n} p_i D_i , \quad \sum_{i=1}^{n} p_i = 1 ,$$

and kurtosis

$$K_0 = 3 \frac{\text{var}(D)}{\bar{D}^2} , \quad \text{var}(D) = \sum_{i=1}^{n} p_i (D_i - \bar{D})^2$$

were given by Jensen et al. (2005).
Figure 1: Model-selection tree for the brain microstructure. The main criterion for moving down the tree is the time-dependence of the diffusion coefficient and kurtosis. The picture of non-exchanging Gaussian compartments, such as the Standard Model of impermeable stick-like axons embedded in a Gaussian extra-axonal space, falls within node 1.1.1, whereas the Kärger model of exchange between Gaussian compartments in node 1.1.2, cf. Eq. (3). If a time-dependent diffusion is observed, the long-time scaling of \( D(t) \) and \( K(t) \) can be used to determine the structural disorder universality class, some of which are sketched within node 2.2.1 and in Fig. 2. The effects of exchange add to the time-dependence of \( K(t) \) and compete with the disorder coarse-graining effects (node 2.2.2).

Node 1.1.2: Exchanging Gaussian compartments

The presence of time-dependence in \( K(t) \) with no time-dependence in \( D \) indicates exchange between Gaussian compartments, while the residual, non-Gaussian intra-compartmental effects are negligible. In this “adiabatic exchange” regime, the Kärger model (KM) (Kärger et al., 1988) originally developed for chemical solutions has been shown to apply to complex tissue environments (Fieremans et al., 2010). In this case, the diffusivity is time-independent and given by Eq. (1), whereas kurtosis decays on the exchange time scale \( t \sim \tau_{ex} \) (Jensen et al., 2005; Fieremans et al., 2010):

\[
K(t) = K_0 \cdot \frac{2 \tau_{ex}}{t} \left[ 1 - \frac{\tau_{ex}}{t} (1 - e^{-t/\tau_{ex}}) \right],
\]

where \( K_0 \equiv K(t)|_{t=0} \) is given by Eq. (2) above, exemplifying that exchange effects can be neglected for \( t \ll \tau_{ex} \). Conversely, for \( t \gg \tau_{ex} \), kurtosis approaches its limit \( K(t)|_{t \to \infty} = 0 \) of a Gaussian medium asymptotically as \( \sim 1/t \). Finite-pulse PGSE generalization of Eq. (3) was found in the \( t \ll \tau_{ex} \) limit (Ning et al., 2018).

The presence of non-exchanging Gaussian compartments within a voxel would add a constant \( K(t)|_{t \to \infty} = K_\infty \) to Eq. (3), whereas the \( t \)-dependent part (3) would then describe exchanging compartments (with a suitably redefined \( K_0 \)). This candidate behavior will be compared to our experimental findings in Section 4.2 and Fig. 7 below.

Node 2: Intra-compartmental microstructure effects

Node 2 of Fig. 1 corresponds to the time-dependence of the diffusion coefficient, or \( D(t) \). In the absence of flow, \( D(t) \), to the best of our knowledge, can only originate from the presence of microstructure, cf. Sections 1.9 and 2 of the review article...
by Novikov et al. (2019) for a detailed discussion. Incomplete coarse-graining of the microstructure manifests itself in non-
Gaussian diffusion; this results in time-dependence of both $D(t)$ and of non-zero higher-order cumulants, i.e., $K(t)$ and beyond.

Node 2.1: Non-physical case

We are unaware of a physical system where the diffusivity is time-dependent and the kurtosis does not depend on time 
(at any time scale): Physically, the former would indicate that the coarse-graining is not over, while the latter corresponds to complete coarse-graining. This contradiction suggests checking 
the processing pipeline with respect to parameter estimation biases but also the calibration of the MRI pulse sequence that is being used. For those types of contradicting results, pulse sequence calibration using an ice-water phantom (Malyarenko et al., 2016; Fieremans and Lee, 2018) is recommended.

Node 2.2: Diffusion in the presence of microstructure

For this general case of both $D(t)$ and $K(t)$ being time-dependent, we will assume the range of diffusion times $t > t_d$ to 
be exceeded the correlation time $t_c$ corresponding to diffusing past 
the correlation length $l_c = L(t_c)$ of tissue microstructure in a 
given compartment. This assumption is reasonable for the 
brain, since the size of typical structural “features” within the 
neuropil (spines, boutons, axon and dendrite diameters) is about 
$1\mu m$, corresponding to $t_c \sim 1\ ms$, while our diffusion time 
range is at least an order of magnitude greater.

While the neuropil generally dominates the cellular volume (Chklovskii et al., 2002), we note that this assumption may be 
invalid in certain individual cortical layers with notable density of 
nearlcy soma. In this case, the diffusion inside the neuronal 
organelles should also be modeled, generally leading to the soma 
contributions to $D(t)$ and $K(t)$ both decreasing as $1/t$ for $t > t_D$ 
as originating from a closed compartment of soma radius $r \sim R$. For $t \lesssim t_D$, $K(t)$ from soma would increase 
with $t$; as below we observe that $K(t)$ decreases monotonically, 
and such short-$t$ contribution seems undetectable in the overall 
$K(t)$ of our in vivo measurements.

Node 2.2.1: Effects of intra-compartmental microstructure; no exchange between compartments, $t_c \ll t \ll t_{ex}$

Coarse-graining the microstructure in a given compartment past the correlation length, i.e., $L(t) \gg l_c$, results in distinct 
power-law tails (Novikov et al., 2014, 2019) in the instantaneous 
diffusion coefficient for this compartment,

$$ D_{\text{inst}}(t) \equiv \frac{1}{2} \partial_t \langle x^2(t) \rangle \approx D_\infty + A \cdot t^{-\theta} \text{.} (4) $$

Here, the dynamical exponent

$$ \theta = (p + d)/2 \text{.} (5) $$

is related to the compartment’s spatial dimensionality $d$, and to 
the disorder universality class, defined in terms of the structural 
exponent $p$ describing long-range density fluctuations $n(x_0)$ of 
the microstructure via its power spectrum:

$$ \Gamma(k) \equiv \int_{V} \langle n(x_0 + x)n(x_0) \rangle_{x_0} = \frac{n(0)^2}{V} \sim k^p \text{, } \quad k \to 0 \text{.} (6) $$

Here $V$ is the compartment volume (or length in $d = 1$), and $n(0)$ is the Fourier transform of $n(x_0)$. In other words, coarse-
grading the structurally disordered microstructure $n(x_0)$ over 
the diffusion length $L(t)$ corresponds to probing the variance 
$\Gamma(k)$ of the structural fluctuations $n(x_0)$ at the corresponding 
wave vector $k \sim 1/L(t)$. In this way, measuring the diffusive 
properties enables probing the degree of spatial correlations of 
microstructural building blocks. The coefficient $A$ in Eq. (4) 
is proportional to that in front of $k^p$ in Eq. (6); we can therefore 
say that $\Gamma(k) \propto A \cdot t^{-\theta}$ as $k \to 0$.

The typically measured cumulative diffusion coefficient

$$ D(t) = \frac{1}{2t} \langle x^2(t) \rangle = \frac{1}{t} \int_0^t D_{\text{inst}}(t')dt' \text{.} (7) $$

will have the same power-law scaling

$$ D(t) \approx D_\infty + c_D \cdot t^{-\theta}, \quad c_D = \frac{A}{1 - \theta}, \text{ for } \theta < 1, \text{.} (8) $$

and will approach $D_\infty$ as $t \to 1/\theta$ for $\theta > 1$ (Novikov et al., 2014). The borderline case of $\theta = 1$ yields the $\ln(t/t_c)/t$ behavior (Burcaw et al., 2015)

$$ D(t) \equiv D_\infty + A \cdot \left( \frac{\ln(t/t_c)}{t} \right), \quad \tilde{t}_c \sim \max\{t_c, \delta\}, \quad \theta = 1, \text{.} (9) $$

where $\delta$ is PGSE pulse width. The $1/(1 - \theta)$ divergence in $c_D$ 
as $\theta \to 1$ can be attributed to $\ln(t_c)$, as described in Appendix A. The $\ln(t/t_c)/t$ behavior is applicable when $t \gg t_c$. For wide gradient 
pulses, i.e. $t \gg \delta \gg t_c$, this functional form is generalized to (Burcaw et al., 2015)

$$ \frac{\ln(t/t_c)}{t} \rightarrow \frac{\ln(t/\delta) + \frac{1}{2}}{t - \delta/3}. $$

We will use this generalized form below for our finite-\$ \delta \$ measurements.

The central theoretical result of this work is the general relation 
between the power law tails in $D(t)$ and $K(t)$ for any $p$ and 
d. Specifically, the same power-law exponent $\theta$ appears in the 
kurtois for $t \gg t_c$:

$$ K(t) \approx K_\infty + c_K \cdot t^{-\theta}, \quad \theta < 1, \text{.} (10) $$

where $K_\infty \equiv 0$ for a single compartment (as diffusion asymptotically 
becomes Gaussian). Moreover, the dimensionless ratio $\xi$ 
of the tails $K(t) - K_\infty$ and $\lfloor D(t) - D_\infty \rceil / D_\infty$, is exactly given in 
terms of $p$ and $d$ (Appendix A):

$$ \xi(p, d) \equiv \frac{c_K}{c_D/D_\infty} = 6 \cdot \left( \frac{2 + p(3p + d - 4)}{2(p + 2)} \right) \cdot \frac{1}{2 - \theta} - 1. \text{ (11) } $$

The borderline case $\theta = 1$ has the same $\ln(t/t_c)/t$ behavior as in 
Eq. (9), with $c_K$ formally diverging as $1/(1 - \theta)$. Appendix A:
Figure 2: Cartoon representation of mapping the complex microstructure onto simpler systems. a) Mapping of dendrites (Glantz and Lewis, 2000) and axons (Shepherd et al., 2002) into a $d = 1$ dimensional transmission line with barriers of permeability $\kappa$ (node 2.2.1.1 in Fig. 1). Here shows an example of caliber variation (blue) along an axon, and the local maxima (red) in caliber are identified as microstructural inhomogeneity along the axon. b) A system of randomly distributed disks in $d = 2$ dimensions (node 2.2.1.2 in Fig. 1). c) A $d = 3$ dimensional system of random rods (node 2.2.1.3 in Fig. 1). The panel a) is adapted from (Glantz and Lewis, 2000; Shepherd et al., 2002) with permission from American Medical Association and National Academy of Sciences of the United States of America.

Beside the theoretical generality of the results (11) and (12), we note that practically, within the limited range of diffusion times in actual experiments, any above functional forms of $D(t)$ and $K(t)$ can fit the measured time-dependence well. The exact result for the tail ratio allows us to further narrow down the choice between the models of structural disorder, instead of just relying on the goodness-of-fit for $\vartheta$. Furthermore, as we see, the same tail in $D(t)$ can originate from distinct $p$ and $d$, in which case the knowledge of an exact tail ratio is essential.

In Sections 3.4, 4.2 and 4.4 below, we will analyze the structural correlations and the temporal scaling laws (8) and (10) for the microstructure in gray matter. Below we consider relevant microstructural arrangements:

- Node 2.2.1.1, diffusion inside narrow long neurites (axons and dendrites), restricted by spines, beads, shafts and other heterogeneities with local density $n(x_0)$, Fig. 2a. Coarse-graining over the diffusion length $L(t)$ exceeding both the typical distance between the restrictions and the neurite diameter (so that the diffusion can be considered one-dimensional) maps the diffusion in a 3-dimensional neurite onto a one-dimensional diffusion with a diffusivity $D(x_0)$ smoothly varying on the scale $\gtrsim L(t)$, whose long-range fluctuations mimic those of $n(x_0)$. In Section 4.4 we will
show that the power spectrum $\Gamma(k)$ of $n(x_0)$, Eq. (6), is
characterized by the structural exponent $p = 0$ as $k \to 0$.
In dimension $d = 1$, this yields $\theta = 1/2$ (Novikov et al.,
2014) and the ratio $\xi(0,1) = 2$ (Dhital et al., 2017), such that
\[ D(t) - D_\infty \approx 2A \cdot t^{-1/2}, \quad \frac{K(t) - K_\infty}{D_\infty} \approx \frac{4A}{D_\infty} \cdot t^{-1/2}. \quad (13) \]

**Node 2.2.1.2.** Diffusion in the extra-neurite space trans-
verse to a coherent randomly-packed fiber bundle, Fig. 2b.
Burcaw et al. (2015) showed that such a neuronal tract
is characterized by short-range disorder, exponent $p = 0$
in dimension $d = 2$, yielding $\theta = 1$, $D(t)$ described by
Eq. (9), and the ratio $\xi(0,2) = 6$, such that
\[ K(t) - K_\infty \approx \frac{6A}{D_\infty} \ln(\frac{t}{\xi_0}), \quad p = 0 \quad \text{in} \quad d = 2. \quad (14) \]

**Node 2.2.1.3.** Diffusion in the extra-neurite space of ran-
domly placed and oriented neurites embedded in a $d = 3-$
dimensional space, Fig. 2c. This is an example of ex-
tended disorder (“random rods”) (Novikov et al., 2014),
for which exponent $p = -1$, such that structural fluctua-
tions diverge. While $D(t)$ has the same form (9) as in Node
2.2.1.2, Eq. (12) yields different $\xi(-1,3) = 42/5$, i.e.
\[ K(t) - K_\infty \approx \frac{42}{5} \frac{A}{D_\infty} \ln(\frac{t}{\xi_0}), \quad p = -1 \quad \text{in} \quad d = 3. \quad (15) \]

The last two nodes exemplify the fact that both disorder classes –
short-range in $d = 2$ and extended in $d = 3$ – create qualita-
tively similar restrictions to diffusion, governed by the dynam-
ical exponent $\theta = 1$. They can be further distinguished by the
tail ratio of $K(t)$ and $D(t)$, Eqs. (11)–(12).

**Node 2.2.2: Competition between intra-compartmental mi-
crostructure and inter-compartmental exchange, $t_\text{c} \ll t_\text{ex} \leq t$**

An interesting case emerges when, while coarse-graining oc-
curs in each compartment, molecules can hop between the com-
partments: that is, the exchange begins to interfere with nontri-
vial intra-compartmental diffusion. While this case has not been
studied quantitatively, qualitative considerations were given in
Appendix F of (Burcaw et al., 2015), arguing that the adiabatic
exchange does not alter the dynamical exponent. In this picture,
the overall diffusivity $D(t)$ will scale with the slowest compart-
mental dynamical exponent $\theta$ provided that $\theta < 1$, and such
intra-compartmental $t^{-\theta}$ scaling will also dominate in the over-
all $K(t)$, since its asymptotic decrease due to the exchange hap-
pens with a power-law tail $K(t) \sim 1/t$, cf. Eq. (3), that de-
cays faster than that in Eq. (10). The logarithmic singularity for
$\theta = 1$ (if such exponent is dominant) will also hold in both
$D(t)$ and $K(t)$, cf. Eq. (12). Finally, for $\theta > 1$, similar con-
siderations predict that $D(t)$ and $K(t)$ will decrease as $1/t$ with
non-universal coefficients, which will not be immediately re-
lated to each other (contrary to Eq. (11)), since the one in $D(t)$
would be dominated by the non-universal short-time behavior
of $D_{\text{ex}}(t)$ according to Eq. (7) (Papaiouannou et al., 2017), while
that in $K(t)$ will have the admixture of exchange, cf. Eq. (3).
For white matter, the intra-extra axonal exchange rate $t_{\text{ex}}^{-1}$
was found to range between $0.3 \sim 1.8 \text{s}^{-1}$ (Lampinen et al.,
2017). For neurons and glial cells grown on polystyrene beads,
the exchange time was recently estimated to be $t_{\text{ex}} \approx 115 \text{ms}$
(Yang et al., 2018). In live and fixed excised neonatal mouse
spinal cord, Williamson et al. (2019) observed the water ex-
change rate $\sim 100 \text{s}^{-1}$ between membrane structures and free
environments. Measurement for diffusion times of the order of
or exceeding 100 ms may thereby be affected by the physics of
exchange.

3. Methods

3.1. Acquisition

Diffusion MRI was performed on 10 healthy volunteers (7
males and 3 females) ranging between 23 to 30 years old
on a Siemens Prisma (3T) system after obtaining a consent which
was approved by the Institutional Review Board. A monopo-
lar PGSE (Siemens WIP 511E) diffusion weighting sequence
was used for acquiring diffusion-weighted images (DWIs) of
four b-shells ($b = [0.1, 0.4, 1.0, 1.5] \text{ms}/\mu\text{m}^2$) along 64 direc-
tions in total. In addition, 2 $b = 0$ images were acquired, one
with phase encoding according to anterior-posterior (AP), the
same as the DWIs, and one addition according to posterior-
 anterior (PA) for distortion correction. The diffusion time,
determined as $t = \Delta$ in the PGSE sequence, was varied as
$21.2 - 22 - 24 - 26 - 28.6 - 35 - 40 - 50 - 75 - 100 \text{ms}$, all
with the same gradient pulse duration $\delta = 15 \text{ms}$. (The approx-
imate equivalence of $t$ with $\Delta$, with its precision determined
by $\delta$, is explained in Section 2.3 of the review by
Novikov et al. (2019).) The remaining experimental parameters of
the sequence are detailed below: TE = 150 ms, TR = 5000 ms,
resolution = $2.0 \times 2.0 \times 2.0 \text{ mm}^3$. A slab of 15 slices was ac-
quired and was aligned parallel to the anterior commissure (AC)
- posterior commissure (PC) line. The total scan time for each
subject was approximately one hour.

The sequence was calibrated using an ice-water phantom
(Malyarenko et al., 2016) at 0°C, resulting in $D_0 = 1.1 \mu\text{m}^2/\text{ms}$
and $K = 0.01$ over a diffusion time range $t = 21.2 - 100 \text{ms}$, ver-
ifying that there is no artificial time-dependence induced in the
diffusion coefficient or kurtosis by the pulse sequence (Supple-
mental Fig. S1). An MPRAGE image was also acquired with res-
olution $= 1.0 \times 1.0 \times 1.0 \text{ mm}^3$, TE = 2.7 ms, TR = 2100 ms,
and used for the gray matter segmentation.

3.2. Data processing

The processing pipeline of the diffusion weighted images
(Ades-Aron et al., 2018) included noise reduction using MP-
PCA (matrix dwidenoise) (Veraart et al., 2016) resulting a
signal-to-noise ratio (SNR) $\approx 35$ in $b = 0$ images, Gibbs ring-
ning removal (matrix mrdegibbs) (Kellner et al., 2016), correc-
tion of susceptibility-induced distortion (FSL topup) (And-
ersonsson et al., 2003), motion and eddy current correction (FSL
eddy) (Andersson and Sotiropoulos, 2016), and Rician noise


Figure 3: Time-dependence of diffusion metrics in human cortical gray matter. Diffusivity reveals a weak and noisy time-dependence, whereas diffusional kurtosis reveals a strong and distinct time-dependence (Both are significant with P-values < 0.05). a) Time-dependence of axial, radial and mean diffusivity for all 10 subjects. b) Time-dependence of mean diffusivity averaged among all subjects. c) Time-dependence of axial, radial and mean kurtosis for all subjects. d) Time-dependence of mean kurtosis averaged over all subjects. Right panel: Cortical gray matter ROI shown on a b = 0 image.

correction (Koay and Basser, 2006). DWIs of all time points were processed jointly using FSL eddy to avoid further coregistrations and interpolations. Standard diffusion kurtosis imaging (DKI) weighted least squares fitting (Veraart et al., 2013) was applied to DWIs for calculating the diffusion and kurtosis tensors. In order to compare the diffusivity time-dependence estimated based on diffusion tensor imaging (DTI) and DKI, standard DTI weighted linear least squares fitting was also applied to DWIs of b-values ≤ 0.4 ms/µm² for diffusion tensor calculations (Basser et al., 1994). The effective b-value for non-diffusion weighted images, \( b_{\text{eff}} \), included the contributions from the imaging and crusher gradients, and it was estimated to be \( b_{\text{eff}} = 0.001 \text{ ms/µm}^2 \) for all measured time points in this study.

To extract regions of interest (ROIs) in gray matter, a \( T_1 \)-weighted MPRAGE image was acquired, and the brain was segmented using FreeSurfer (Dale et al., 1999; Destrieux et al., 2010). The labels map in \( T_1 \)-weighted image space was coregistered to the \( b = 0 \) image space using affine transformation (FSL FLIRT) (Jenkinson and Smith, 2001), initialized with the sform/qform in the DICOM header, and was downsampled by using nearest neighbor. The resulting cortical ROI is shown in Fig. 3 in red along with the \( b = 0 \) image. To avoid white matter partial volume effects, the thresholds of fractional anisotropy FA < 0.3 and < 0.4 were respectively imposed to the cortical and deep gray matter ROIs based on previous studies (Alexander et al., 2007; Pfefferbaum et al., 2010). Further, to avoid cerebrospinal fluid (CSF) signal contamination, voxels close to CSF were excluded using a CSF mask generated by FSL FAST (Zhang et al., 2001), and was expanded by one voxel. Lastly, the diffusion coefficient and diffusion kurtosis for each time point and subject was calculated by averaging over all voxels of the parametric maps in the cortical gray matter ROI (Fig. 3a and 3c) and in each gray matter sub-region (Fig. 5).

To compare our results with the diffusivity time-dependence observed in white matter by Fieremans et al. (2016), white matter ROIs were also segmented by transforming John’s Hopkins University DTI-based white matter atlas (Mori et al., 2005) to the individual DWI space, as in (Fieremans et al., 2016).
Figure 4: The time-dependence of diffusion kurtosis appeared consistent between subjects. a) time-dependence of mean kurtosis for all subjects. b) Goodness of fit according to Eq. (10) for all subjects scanned in this work. It is observed that averaging over all subjects improves the quality of the fit substantially. c) Fitted dynamical exponent $\vartheta$ for all subjects scanned in this work. d) Fitted coefficient $c_K$ was found to have moderate variation between subjects. e) Fitted $K_\infty$ for all subjects scanned in this work.

3.3. Parameter estimation

The three-parameter power-law relations (8) and (10) were fitted to measured time-dependent mean diffusivity and kurtosis. The weighted non-linear least square fit was initialized with 1000 different combinations of initial values, and the largest cluster in parameter space was identified by using density-based clustering (Ester et al., 1996). We chose the median of fitted parameters within the cluster to determine the exponent $\vartheta$.

To stabilize the three-parameter power-law fitting, the weight for each $t$-point was determined via Rician MRI noise propagation through DKI pipeline, as follows: For one specific $t$-point, we applied Rician noise to the denoised DWIs based on the estimated noise map (Veraart et al., 2016), performed DKI estimation, and repeated this procedure for 10 times to calculate the variance of estimated diffusivity and kurtosis between different noise realizations. The error bars for all figures was the square root of mean variance within each ROI, manifesting the noise propagation of DKI estimations. Further, we calculated weights for fitting using the inverse of mean variance within each ROI.

To evaluate the strength of the mean diffusivity and kurtosis time-dependence, we hypothesized that $D(t)$ and $K(t)$ are linear functions of $t^{\vartheta}$ based on Eqs. (8) and (10) and the estimated $\vartheta$, and calculated statistical $P$-values with the null hypothesis of being no positive correlation (one-sided test). The significance level was set at 0.05 for the overall cortical gray matter, and was set at 0.002 for each gray matter sub-region (Bonferroni correction for 25 sub-regions).

3.4. Structure correlation function of axonal beading

To investigate the structure of axons in gray matter (Node 2.2.1.1 in Fig. 1), we processed the data of axonal bead locations (“swellings” coinciding with synaptic boutons) in mouse cortex, originally published by Hellwig et al. (1994). This work reports on the bead locations of 33 axons of different length, $L_m$ ($m = 1...33$), ranging from approximately 100 $\mu$m to 400 $\mu$m.
The construction of the power spectrum $\Gamma(k)$, Eq. (6), was performed according to following three steps:

1. The axonal bead density, $n(x)$, was digitized and concatenated into a single, digitized axonal line of length $L$. Note that $L \gg L_m$.

2. The procedure of concatenation was repeated 200 times by randomly reshuffling the 33 axons. This procedure creates different disorder realizations.

3. The power spectrum for each disorder realization was computed according to Eq. (6), with $V \rightarrow L$, and the Fourier transform of bead density $\tilde{n}(k) = \int dx e^{-i k x} n(x)$. This power spectrum was then averaged over all disorder realizations. Note that randomly reshuffling the axons and concatenating them reduces the noise fluctuations in $\Gamma(k)$. However, after approximately 200 averages the system becomes aware of the reshufflings, and averaging over subsequent reshufflings does not result in additional noise reduction in $\Gamma(k)$ (Papaiouannou et al., 2017).

### 3.5. MC simulations

Monte Carlo (MC) simulations of Brownian motion in $d = 1$ dimensions with barriers of fixed permeability $\kappa$ were performed, following a toy model of disordered axons in Fig. 2a, corresponding to the Node 2.2.1.1. The “barriers” are meant to describe, e.g., the restrictions by the narrow shafts in-between the beads (cf. Section 5 for discussion).

The barriers were distributed in spatial dimension $d = 1$ according to a PDF $P(\alpha)$ of independent successive intervals $\alpha$, with an average spacing between the barriers $\bar{\alpha} = 4.45 \mu m$ and its variance $\sigma^2_\alpha = 16.4 \mu m^2$, corresponding to short-range disorder, as described by Novikov et al. (2014), Supplementary Information. These microstructural parameters were taken to be similar to those derived from histology (Hellwig et al., 1994) (as described above).

A total of five short-range disorder realizations were simulated. The barriers were distributed on a line of length $L = 7,200 \mu m$ each and approximately 1,600 barriers (restrictions) for each realization. The number of random walkers simulated for each realization was $N = 1 \times 10^8$. The time-step duration for each random walker was $\delta t = 0.002$ ms corresponding to a spatial step size $\delta x = \sqrt{2D_0 \delta t} \approx 0.020 \bar{\alpha}$, with the intrinsic diffusion coefficient $D_0 = 2 \mu m^2/\mu s$. The initial positions of random walkers are randomly determined in each realization to initialize a constant/homogeneous density.

We simulated membrane permeability in finite-step Monte Carlo according to Appendix B. The probability to cross a barrier was given by Eq. (B.2) with the initial barrier permeability set to $\kappa_0 = 0.4154 \mu m/\mu s$, such that the genuine permeability corrected for the finite step-time $\delta t$ of the simulation was $\kappa = 0.4233 \mu m/\mu s$ (see Appendix B and Eq. (B.8)). This value was chosen to mimic the tortuosity limit corresponding to the membrane “effective volume fraction” (Novikov et al., 2011) $\zeta \approx 1.062$ for all MC simulations. For this model system, Novikov et al. (2014) found the coefficient

$$A = \frac{D_0 \sqrt{\tau_r}}{\sqrt{2\pi}} \cdot \frac{\sigma_D^2}{\bar{\alpha}} \left( \frac{\zeta}{1 + \zeta} \right)^3, \quad c_D = 2A, \quad (17)$$

entering Eq. (8), where $\tau_r = \bar{\alpha}/2\nu$ is the mean residence time within a typical interval between barriers.

The maximum diffusion time was approximately 1300 ms corresponding to 250$\tau_r$. The simulated diffusivity and kurtosis were calculated based on the moments of diffusion displacements, $\langle x^2 \rangle$ and $\langle x^4 \rangle$. The random number generator used was Philox4x32-10 (Salmon et al., 2011) and the MC script was developed in CUDA C++. MC simulations were performed on the New York University BigPurple high-performance-computing cluster, and the total calculation time was 60 min using 5 GPU cores.

To evaluate the bias due to the imaging protocol and kurtosis fitting, we also simulated diffusion signals of narrow pulse with $b$-values $= [0.1, 0.4, 1, 1.5] \mu m^2/\mu s$ as in experiments, and fitted DKI to signals to estimate diffusivity and kurtosis.

### 3.6. Data and code availability

All human brain MRI data for this study are available upon request. The source codes of image processing DESIGNER pipeline, power spectrum analysis, and Monte Carlo simulations can be downloaded on our github page (https://github.com/NYU-DiffusionMRI).

### 4. Results

#### 4.1. $D(t)$ and $K(t)$ in human gray matter

Figs. 3a and 3c highlights the resulting axial, radial and mean diffusion coefficient and diffusion kurtosis of cortical gray matter for all subjects and time points studied in this work. The noise variance $\sigma^2_D$ of both diffusion coefficient and kurtosis of the cortical ROI was similar for each subject and for each time point, and was approximately $\sigma^2_D \approx 0.001 \mu m^2/\mu s$ and $\sigma^2_K \approx 0.005$ indicating reasonable noise propagation of DKI estimation. The observed fractional anisotropy (FA) values for the cortical ROI were approximately 0.18, indicating a small anisotropy between diffusion directions; this observation allows us to focus on the mean values of the tensor diffusion metrics.

By performing an average of the mean diffusivity and kurtosis over all subjects, a distinct time-dependence was observed in the diffusion kurtosis at the time scale of the experiment as shown in Fig. 3d. The observed fractional anisotropy (FA) values for the cortical ROI were approximately 0.18, indicating a small anisotropy between diffusion directions; this observation allows us to focus on the mean values of the tensor diffusion metrics.

The comparison of DTI and DKI results showed that, while DKI yielded slightly larger diffusivity values than DTI in most of the brain ROIs, the diffusivity time-dependence given by DTI and DKI was nearly identical (data not shown). Furthermore,
in brain white matter ROIs, we also observed significant axial and radial diffusivity time-dependence in this dataset, consistent with the previous study (Fieremans et al., 2016).

4.2. Estimation of dynamical exponent $\theta$ (Node 2.2.1)

Eq. (10) was used to estimate the observed dynamical exponent from the subject-averaged mean kurtosis. The result was $\theta = 0.56$ after performing a three degrees of freedom least squares fit, with $c_K = 0.70$ and $K_{\infty} = 0.68$, and $\chi^2/\text{DOF} = 1.04$.

Fig. 4a shows the mean kurtosis for all subjects scanned in this work along with the statistics of the three degrees of freedom parameter fit to Eq. (10). Relatively high $\chi^2/\text{DOF}$ was observed for each fit in comparison to the global, as shown in Fig. 4b. On the other hand, reasonable agreement was observed between the fitted values $c_K$ and $K_{\infty}$ of each subject (Fig. 4c-d-e).

Fig. 5 highlights the scaling of mean kurtosis for 25 additional ROIs of sub-regions in deep and cortical gray matter, in comparison with the global cortical gray matter. A reasonable agreement was observed between the global dynamical exponent $\theta = 0.56$ and $\theta$ for each ROI in Fig. 6.

Fig. 7 shows the measured mean kurtosis of the global cortex with respect to $t^{-0.5}$, $t^{-1}$, $[\ln(t/\delta) + 3/2]/(t - \delta/3)$, and $t$. A straight line was observed in Figure 7a-b when kurtosis is plotted with respect to both $t^{-0.5}$ and $t^{-1}$, with $\chi^2/\text{DOF} \sim 0.9$ and 1.4 respectively. In addition, a straight line was observed in Fig. 7c when kurtosis is plotted with respect to $[\ln(t/\delta) + 3/2]/(t - \delta/3)$ (Burcaw et al., 2015) with $\chi^2/\text{DOF} \sim 1.1$. This observation reveals that the fit does not allow for a statistically confident model selection between the three functional forms.

Instead, we can select models in Node 2.2.1 by comparing the time-dependence of diffusivity and kurtosis, i.e. the ratio $\xi$ of $c_K$ to $(c_D/D_0)$ in Eq. (11). In Fig. 7a and 7c, the ratio $\xi = 2.43$ and 2.41 for $t^{-0.5}$ and $[\ln(t/\delta) + 3/2]/(t - \delta/3)$ power-law indicates that the short-range disorder in 1d ($\xi = 2$) is the most preferred model in Node 2.2.1. We discuss these findings further in Section 5.

4.3. Kärger model’s parameter estimation (Node 1.1.2)

If we were instead to adopt the exchange picture between Gaussian compartments, fitting the KM kurtosis (3) to the observed mean kurtosis would yield an exchange time between compartments, which are most likely to be the neurites (dendrites and axons) and the extra-neurite space. Fig. 7d shows the measured mean kurtosis and the fit of Eq. (3) (with the added constant $K_{\infty}$) to the data in black dashed line. The fit had a
\[ \chi^2/\text{DOF} = 0.97 \] and an exchange time value \( t_{\text{ex}}^{\text{KM}} \approx 11 \text{ ms} \). On the other hand, a fit of the original Kärger Model (setting \( K_\infty \equiv 0 \)) yields an exchange time of \( t_{\text{ex}}^{\text{KM}} \approx 250 \text{ ms} \) with a relatively poor \( \chi^2/\text{DOF} \approx 3.2 \). The above estimated exchange times, either with or without a non-zero \( K_\infty \), are out of the range of our measurements with diffusion time \( t = 21.2 - 100 \text{ ms} \).

4.4. Structure correlation function of axons in gray matter

We now study the low-\( k \) behavior of the power spectrum Eq. (6) of bead placement density \( n(x_0) \) quantified from the measurements by Hellwig et al. (1994) in mouse cerebral cortex, to determine the structural exponent. It is useful to consider the dimensionless \( \Gamma(k) \cdot \hat{a} \), which is equal to unity for Poissonian statistics, as shown in Fig. 8a (blue) for simulated fully uncorrelated barrier placement.

For general short-range disorder, residual correlations give rise to a plateau in \( \Gamma(k)_{|k|<\hat{a}} \cdot \hat{a} \) different from unity. Based on histology, a plateau of approximately 0.6 was observed after constructing the structure correlation function (6) shown in Fig. 8a in red for the bead placements of (Hellwig et al., 1994). This indicates that bead occurrence along axons corresponds to a short-range disorder, confirming the structural exponent \( p = 0 \) announced in Node 2.2.1.1, Section 2. In addition, Fig. 8b highlights the PDF \( P(\hat{a}) \) of the successive intervals for artificially constructed Poissonian disorder, and for the experimentally measured axonal bead placements from (Hellwig et al., 1994). Although noisy, a maximum of the PDF for the bead placement (in red) at \( a/\hat{a} \approx 0.6 \) may distinguish it from the perfectly exponential PDF \( = (1/\hat{a}) \cdot e^{-a/\hat{a}} \) (linear in semilogarithmic scale) for the Poissonian statistics (blue).

An alternative approach for distinguishing Poissonian statistics is investigating the scaling of the mean number of restrictions \( \langle N \rangle \) within a window of length \( L_p \) with respect to their variance \( \langle N^2 \rangle - \langle N \rangle^2 \) within this window (Shepherd et al., 2002). Fig. 8c shows such scaling. For Poissonian statistics, \( \langle N^2 \rangle - \langle N \rangle^2 = \langle N \rangle \) is expected, as shown by the blue line. On the other hand, the solid red lines represent this scaling for the 33 individual axons measured in (Hellwig et al., 1994) along with a fit over all axons shown by the dashed line. As expected, for short-range disorder, \( \langle N \rangle \) grows in proportion to \( \langle N^2 \rangle - \langle N \rangle^2 \) but with a slope \( \approx 0.73 \) different from 1.

What is short-range disorder qualitatively, and why is it ubiquitous? The hallmark of short-range disorder is the finite correlation length \( l_c \), beyond which the correlation function \( \langle n(x_0 + x)n(x_0) \rangle_{x_0} \) decays sufficiently fast (this applies in any dimension, not just in \( d = 1 \)), so that the “memory” about where one should expect another restriction is forgotten for \( x \gg l_c \). In other words, for such large \( x \), one could view the correlation function \( \sim \delta(x) \) as a \( \delta \)-function of the width \( \sim l_c \). Hence, in the \( k \)-space, the power spectrum of such a localized object is approximately constant, \( \Gamma(k) \sim k^p \) \( = \text{const} \) for all \( k \lesssim 1/l_c \), yielding the structural exponent \( p = 0 \).
Figure 7: Model comparison for \( d = 1 \), \( d = 2 \) and \( d = 3 \) structural disorder classes, and for the Käger model. a) Mean kurtosis \( K(t) \) in cortical gray matter plotted as a function of \( t^{-0.5} \). b) \( K(t) \) plotted as a function of \( t^{-1} \). c) \( K(t) \) plotted as a function of \( \frac{\ln(t/\delta) + \frac{3}{2}}{t - \delta/3} \). d) Mean kurtosis along with the Käger model fit with \( K_{\infty} \equiv 0 \) in blue, and the Käger model with an added constant \( K_{\infty} \) as black dashed line. All the fit results are summarized in Section 4.2 and 4.3. The residuals between the fit curves and measured data are shown in the bottom row.

We note that other alternatives for the placement of the restrictions are the hyperuniform disorder, \( p > 0 \), with “almost-periodic” placements of the restrictions (characterized by the suppressed structural fluctuations at large distances) that emerge, e.g., due to effective repulsion of restrictions, or can be artificially created (Papaioannou et al., 2017); and the so-called strong disorder, with \( p < 0 \), such that the power spectrum (6) diverges at \( k \to 0 \) (Novikov et al., 2014).

4.5. MC simulations in \( d = 1 \): Diffusion metrics
To investigate the sensitivity of the diffusion coefficient and diffusion kurtosis to the microstructural features and validate Eq. (13), we performed Monte Carlo simulations in dimension \( d = 1 \). Fig. 9a-b highlighted the time-dependence of the diffusion coefficient and diffusion kurtosis up to \( t = 250\tau_r \), and both metrics reached the tortuosity limit already by that time. Diffusivity approached the tortuosity limit, \( D_{\infty} = 0.97 \mu m^2/ms \), for times \( t \gg \tau_r \), where diffusion become effectively Gaussian. Similarly, \( K(t) \) approached zero for \( t \gg \tau_r \). In addition, the kurtosis showed a non monotonic time-dependence at approximately \( t \lesssim \tau_r \), where a maximum was observed as shown in the inset of Fig. 9b.

Fig. 9c shows the simulated diffusivity and kurtosis as a function of \( \sqrt{\tau_r/t} \), so that a straight line is formed at long times according to Eq. (13). Good agreement was observed between Eq. (13) and the simulated diffusivity and kurtosis at long times. It is observed that the system is in the long-time limit at already \( t = \tau_r \) for diffusivity and \( t \approx 4\tau_r \) for kurtosis (insets of Fig. 9a-b), which effectively means that the molecules then already have traversed a couple of mean barrier spacings \( \bar{a} \).

In addition, Fig. 9c highlights the simulated diffusivity and kurtosis along with the theoretical prediction Eqs. (16)–(17) for the slope \( 2A/D_{\infty} \) and \( 4A/D_{\infty} \) (dotted lines). The scaling of the diffusion kurtosis for long times reveals that the system is at the long time limit at approximately \( t \gtrsim 4\tau_r \), which may point to both diffusivity and kurtosis being equally robust metrics. How-
Figure 8: Short-range disorder is revealed in cortical gray matter. a) Power spectrum, \( \Gamma(k) \cdot \tilde{\alpha} \) calculated via Eq. (6), of axonal beadings in the cortex based on (Hellwig et al., 1994) (red) shows a plateau lower than unity as \( k \to 0 \). Power spectrum for the strictly Poissonian disorder is also shown for comparison (blue), with a unity plateau as \( k \to 0 \). b) The corresponding histogram of bead distance in cortex (red) and strictly Poissonian disorder (blue). c) Scaling of number of axonal beadings within a varying window with respect to the variance for each of thirty-three axons taken from (Hellwig et al., 1994). The dashed red line indicates a linear fit of all the red lines with a slope of 0.73, which diverges from the unity line corresponding to the simulated Poissonian disorder (blue).

Figure 9: Simulated diffusivity and kurtosis on a one-dimensional short-ranged disorder line along with theoretical predictions Eqs. (13) and (16)–(17). Diffusivity and kurtosis reveal that the long-time limit starts at \( t \approx \tau_r \) and \( t = 4\tau_r \) respectively. a) Simulated diffusivity with respect to \( t/\tau_r \) approaches its tortuosity value \( D_\infty \). Inset: Normalized diffusivity along with the theory (dashed line) given by Eqs. (13) and (16)–(17). b) Similarly, the simulated kurtosis with respect to \( t/\tau_r \) approaches zero at long times but shows a non-monotonic behavior at \( t \leq \tau_r \). Inset: Short-time limit of diffusion kurtosis shows an initial increase and a plateau at \( t \leq \tau_r \) before reaching the long-time limit for \( t > \tau_r \) agreeing with the theory (dashed line) given by Eqs. (13) and (16)–(17). c) Simulated diffusivity and kurtosis (solid lines) plotted with respect to \( \sqrt{\tau_r/t} \) approach a straight line for \( t \gg \tau_r \), where the long-time limit empirically starts. The two-fold difference in the coefficients \( c_K \) and \( c_D/D_\infty \) from Eq. (13) is apparent in the two-fold difference in the slopes of the simulated quantities for \( t \gg \tau_r \).

However, kurtosis \( t \)-dependence is observed to be relatively twice more pronounced than that of the diffusivity tail \( (D(t)-D_\infty)/D_\infty \) due to the two-fold difference in the coefficients following from Eq. (13). In simulations, at approximately \( t \geq 4\tau_r \), the tails in diffusivity and kurtosis are indeed observed to differ by a factor of 2 (Fig. 9c).

It is not unexpected that in cortical gray matter and for the shortest diffusion time \( t > 20 \) ms studied in this work, the diffusion is effectively in the long time limit, since spines are placed in dendrites with mean spacing \( \tilde{\alpha} \approx 3 - 3.4 \) \( \mu \)m (Glantz and Lewis, 2000), and beads are placed in axon collaterals with \( \tilde{\alpha} \approx 2.4 - 7.5 \) \( \mu \)m (Hellwig et al., 1994). Another important observation extracted from MC simulations is that kurtosis may be the more sensitive metric for observing subtle effects such as time-dependence in cortical gray matter.

Further, the simulation of signals compared to moments revealed that DKI fitting yields a small bias in diffusivity (< 0.1% bias in \( D_\infty \) and 3% in \( c_D \)) and a moderate bias in kurtosis (14% bias in \( c_K \), with the same \( t^{-0.5} \) functional form valid at the same time scale (data not shown).
5. Discussion

In this study, we provided experimental evidence of time-dependent kurtosis in human gray matter at time-scales from 21.2 – 100 ms, whereas diffusivity showed relatively weak and noisy time-dependence during the same time scales. Here, we discuss the interpretation of the observed time-dependence in diffusion kurtosis based on the scenarios introduced in Section 2, and connect them with the underlying microstructure of neurites in gray matter.

Diffusion in gray matter intra-axonal space may be hindered by spines and beads along dendrites and axons which occur at length scales of approximately $3 - 6 \mu m$. Diffusion along the neurites may be modeled as that along one dimensional structurally-disordered channels as shown in Fig. 2a. In this scenario, which corresponds to node 2.2.1.1 of Fig. 1 (cf. Section 2), the time-dependent kurtosis should scale with a power-law of $\theta = 1/2$. A three degrees of freedom fit of Eq. (10) to the experimental data revealed a power-law of $\theta = 0.56$ with a reasonable $\chi^2$/DOF = 1.04, which is sufficiently close to the theoretical $\theta = 1/2$. Therefore, with negligible water exchange between intra- and extra-neurite spaces and low extra-neurite volume fraction (cf. 20% in adult rat cortex (Bondareff and Pysh, 1968)), the measured signal may be originating primarily from the intra-neurite space (at least in areas of gray matter that are not dominated by the cell bodies), pointing towards 1d short-range disorder.

This scenario is also consistent with histology when analyzing the axonal bead placement in gray matter. The class of disorder, i.e. the statistics of restriction placement, may have an effect on the observed time-dependence. Short-range disorder is generally ubiquitous in Physics and in Biology, hence the structural exponent $p = 0$ is not unexpected. The short-range character of restriction placement is supported by the experimental data of $\Gamma(k)$ at low-$k$ values, shown in Fig. 8, suggesting that beads along axons are distributed according to a PDF with a finite mean and variance according to short-range disorder ($p = 0$). In addition to the beads, Morales et al. (2014) also observed that dendritic spines in adult human neocortex are mostly randomly positioned, further supporting the character of short-range disorder in gray matter.

The observed value of diffusivity at the tortuosity limit of Fig. 3 is approximately $D_{\infty} \approx 0.97 \mu m^2/ms$, which allows us to provide an estimate of the permeability of the one-dimensional “barriers” (e.g., shafts between neurite beads) after mapping their complex structure onto a $d = 1$ dimensional transmission line of barriers of permeability $\kappa$. As mentioned earlier, $\bar{a} = 3 \mu m$ for the spines and beads along neurites, which combined with $D_{\infty} = 0.97 \mu m^2/ms$ (this study) and $D_0 \approx 2 \mu m^2/ms$ (Novikov et al., 2018), results in $\zeta \approx 1.06$ and $\kappa \approx 0.63 \mu m/ms$ based on Eq. (16).

The above 1d picture was first introduced by Novikov et al. (2014) to reveal and interpret the $\omega^0$ scaling of the oscillating-gradient diffusion measurement of (Does et al., 2003) in rat cortical gray matter, for which $\theta = 1/2$ was found. It is remarkable that the same power law exponent $\theta = 1/2$ is here observed in human cortical gray matter. Together with our direct quantification of mouse cortical structural disorder from (Hellwig et al., 1994), this suggests that

(i) the $p = 0$ short-range disorder in one dimension is a universal microstructural signature of structural heterogeneity in neurites across mammals; and

(ii) it manifests itself in the $t$-dependent dMRI signal acquired over macroscopic voxels in vivo, and hence, can be quantified and monitored in disease, development and aging.

Another possible scenario is hindered diffusion in the extra-neurite space, which is abundant with cells, ions and metabolic substrates (Nicholson and Phillips, 1981). Extra-neurite space can be modeled as a two- or three-dimensional random medium (depending on its anisotropy), such that the diffusion is restricted either transverse to a fiber bundle (two-dimensional geometry, cf. Fig. 2b), or in 3d by the randomly placed “rods” (cf. Fig. 2c). As discussed in Section 2, the microstructure of each compartment and the dimensions will have an effect on the observed time-dependence of the diffusion metrics. Diffusion in the extra-neurite space would yield a power-law exponent of $\theta = 1$ with a logarithmic singularity in the kurtosis $K(t) \sim \ln(t/\bar{t})/t$ in the case of $d = 2$ and $p = 0$ (short-range disorder) as well as $d = 3$ and $p = -1$ (extended disorder). In cases where $p > 0$, the kurtosis would scale as $\sim 1/t$ at long times. Plotting the experimental data with respect to $1/t$ and $\ln(t/\bar{t})/t$ did not reveal any important features that may point to one scenario or the other as the least squares fits were equally reliable (see Fig. 7 and section 4.1). However, the ratio $\xi = 2.4$ between the tails in $K(t)$ and $D(t)$, in both Fig. 7a and 7c, cf. Eq. (11), is much closer to $\xi = 2$ than to $\xi = 6$ or 42/5. This ratio further indicates that 1d short-range disorder ($\theta = 1/2$ and $\xi = 2$), originating from the intra-neurite space, is the most preferred model in Node 2.2.1. The estimated ratio $\xi$ is not exactly 2 probably due to contributions of diffusivity and kurtosis time-dependence in other compartments, e.g., extra-neurite space and astrocytes, with different (and non-dominant) power-law exponents. To sum up, for the first time, the comparison of diffusivity and kurtosis time-dependence ($c(t)/D_{\infty}$ and $\kappa(t)$) reveals the structural disorder in tissue micro-geometry.

The last scenario to be discussed is that of exchange, here approximated by the Kärger Model. KM with a non-zero $K_\infty$ yields an exchange time $t_{\chi}^{KM} \approx 11$ ms ($\chi^2$/DOF = 0.97), contradicting the underlying assumption of slow exchange regime (Fieremans et al., 2010). Furthermore, a fit of the original KM (setting $K_\infty \equiv 0$) yields an exchange time $t_{\chi}^{KM} \approx 250$ ms with a relatively poor fit quality ($\chi^2$/DOF = 3.2). Both exchange time estimates, using KM with or without $K_\infty$, are out of the range of our measurements ($t = 21.2-100$ ms), hence their reliability cannot be high. More importantly, significant diffusivity time-dependence was observed in cortical gray matter, inconsistent with an expected time-independent diffusivity in KM. Hence, we conclude that KM and related exchange cannot be used to explain the observed diffusivity and kurtosis time-dependence.

At the same time, however, we cannot exclude a possible contribution of exchange (as a physical effect, beyond a relatively primitive KM) to our observed data. While exchange...
time \( \tau_{es} > 1000 \text{ ms} \) was in vivo measured in lenticular nucleus and thalamus using FEXI (Lampinen et al., 2017), and \( \tau_{ex} \approx 115 \) ms was found between extra-neurite space and astrocytes in vitro (Yang et al., 2018), much shorter exchange time range \( \tau_{ex} \approx 10-30 \text{ ms} \) was recently found in human gray matter on a human Connectome scanner in the high-\( b \)-regime, at \( b \lesssim 25 \text{ ms/μm}^2 \) (Veraart et al., 2018). Furthermore, exchange times of about 10 ms were found in live and fixed excised neonatal mouse spinal cord between membrane structures and free environments using DEXSY (Williamson et al., 2019). Therefore, we speculate that human gray matter may be in the crossover regime, where the exchange effects compete with those of the structural disorder (Node 2.2.2); in this picture, exchange is likely to affect the numerical coefficients, such as \( D_{\omega_0} \), \( c_D \) and \( c_K \), whereas the qualitative \( t^{-1/2} \) power-law scaling is determined by the structural disorder.

A few experimental limitations may not allow us to extract more accurate exchange times and parameters of the scaling laws from the data. First, a larger diffusion time window is necessary in order to accurately capture the power-law dependence of Eqs. (8) and (10), as well as to fit the Kärger Model. While a \( T_1 \)-weighted sequence of the type of STEAM allows for longer diffusion times, it may also introduce artificial time-dependence in the diffusivity and kurtosis due to molecular exchange between compartments (e.g., myelin water and intra/extra-cellular water in white matter) during the STEAM storage times (Lee et al., 2017). To rule out the latter confounding factor, we used spin-echo sequence with fixed TE and TR to fix the \( T_1 \)-weighting and exchange effect between compartments. In addition, a smaller voxel size may be beneficial in order to allow for a more accurate ROI selection and better statistics in the estimation of the diffusion coefficient and kurtosis. A possible approach to simultaneously evaluate water exchange and structural disorder (Node 2.2.2) is to extend the effective medium theory to other more advanced sequences/diffusion gradient waveforms (Jespersen et al., 2019), such as the FEXI sequence (Lasić et al., 2011).

6. Conclusions

In this work, time-dependent kurtosis was observed for the first time in human gray matter at time scales \( t = 21.2-100 \text{ ms} \). Using the proposed model selection tree of Fig. 1 for time-dependent diffusivity and kurtosis, we conclude that 1\text{d}\ structural disorder along the one-dimensional neurites plays the dominant role. The estimated dynamical exponent \( \theta \approx 1/2 \) suggests that diffusion along neurites is affected by short-range disorder (randomly positioned restrictions), consistent with histological results, and the observed power-law is different from that of the Kärger model (3), \( K(t) \sim 1/t \) in long time limit \( (t \gg \tau_{ex}) \). Furthermore, the exchange time \( (\approx 11 \text{ ms}) \) given by Kärger model is out of our measurement range, as well as contradicting the KM assumption of slow exchange regime. Therefore, the observed time-dependence occurs due to physics beyond the KM. Exchange may contribute to the observed \( D(t) \) and \( K(t) \), such that the diffusion in human gray matter at these time scales may be in the crossover regime, where the exchange competes with the structural disorder (Node 2.2.2 in selection tree), while the disorder sets the overall \( t^{-1/2} \) scaling of \( D(t) \) and \( K(t) \). In conclusion, while model-selection is not fully resolved, we present a compelling case of the sensitivity of time-dependent dMRI to the structural disorder along the neurites in the gray matter.

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Appendix A. Relation between power-law tails in \( D(t) \) and \( K(t) \)

Diffusion in the long time limit \((t \gg t_c)\) effectively homogenizes a sample’s microstructure (Novikov et al., 2014), mapping the problem onto that characterized by a smoothly varying local diffusivity \( D(x_0) \) with a mean \( \bar{D} \) and a small variation \( \delta D(x_0) = D(x_0) - \bar{D} \) relative to the mean (Novikov and Kiselev, 2010). The crucial observation is that the spatial fluctuations of \( D(x_0) \) mimic those of the microstructural restrictions \( n(x_0) \) at large displacement \( x \); in particular, the power spectrum

\[
\Gamma_D(k) = \frac{D(-k)D(k)}{V} \approx B \cdot k^p, \quad k \to 0
\]

is characterized by the same structural exponent \( p \) as in Eq. (6).

In what follows, we will relate the power-law tails in \( D(t) \) and \( K(t) \) to the effective medium parameter \( B \) of Eq. (A.1), based on the perturbative treatment up to the order \( O(\delta D^2) \), i.e., up to the first order in the power spectrum (A.1). Our starting point is the cumulants of molecular displacements, given by Eqs. (24)–(25) of (Novikov and Kiselev, 2010):

\[
\langle x^2 \rangle = 2! \int \frac{d\omega}{2\pi} e^{i\omega t} \frac{\mathcal{D}(\omega)}{(-i\omega_x)^2}, \quad \langle x^4 \rangle = 4! \int \frac{d\omega}{2\pi} e^{i\omega t} \left[ \frac{\Sigma_i(\omega)}{(-i\omega_x)^2} + \frac{\mathcal{D}^2(\omega)}{(-i\omega_x)^2} \right],
\]

with \( \Sigma_i(\omega) \) explained later. The symbol \( \omega_x \) denotes that the integration is calculated on a complex plane of \( \omega \), and all poles are in the lower half-plane as a result of causality, cf. Appendix A of (Novikov et al., 2019).
The dispersive diffusivity in Eqs. (A.2)–(A.3) is given by Eq. (7) of (Novikov et al., 2014)
\begin{equation}
D_0(\omega) - D_{\infty} = \frac{-\imath \omega}{d D_0} \int \frac{d^d k}{(2\pi)^d} \frac{\Gamma_D(k)}{\imath \omega + D_\omega k^2} \tag{A.4}
\end{equation}
equivalent to the instantaneous diffusion coefficient (4)
\begin{equation}
D_{\text{inst}}(t) - D_{\infty} \approx \frac{1}{d D_0} \int \frac{d^d k}{(2\pi)^d} \Gamma_D(k) e^{-D_{\omega} k^2 t} = A \cdot t^{-\theta} \tag{A.5}
\end{equation}
where, for \( \Gamma_D(k) \) from Eq. (A.1), we obtain
\begin{equation}
A = \frac{B \cdot \Omega d \cdot \Gamma_E(\theta)}{2d \cdot (2\pi)^d \cdot D_0^{2\theta}} \tag{A.6}
\end{equation}
Here, \( \Omega_d = 2d^{d/2}/\Gamma_E(d/2) \) is the surface area of a unit sphere in \( d \) dimensions \((\Omega_d = 1, 2\pi, 4\pi \text{ for } d = 1, 2, 3)\), and \( \Gamma_E(\cdot) \) is Euler’s \( \Gamma \)-function. Using either Eq. (A.4) or the relation \( D_0(\omega) = -\imath \omega \int e^{\imath \omega D_{\text{inst}}(t)} dt \) (cf. Section 2.2.2 of (Novikov et al., 2019)), we find in the frequency domain
\begin{equation}
D_0(\omega) \approx D_{\infty} + A \Gamma_E(1 - \theta) \cdot (-\imath \omega)^{\theta} \tag{A.7}
\end{equation}
Hence, using Eq. (7), we find
\begin{equation}
\langle x^2 \rangle = 2d D_0(\omega) t \approx 2D_{\infty} t + 2CD_0 t^{1 - \theta} \tag{A.8}
\end{equation}
and \( CD \) is defined in Eq. (8).

As we can see from Eq. (A.3), the dispersive diffusivity alone is not enough to calculate the kurtosis. We will now show that, in general, the fourth order dispersive kinetic coefficient, Eq. (33) of (Novikov and Kiselev, 2010),
\begin{equation}
\Sigma_{d}(\omega) = \int \frac{d^d k}{(2\pi)^d} \Gamma_D(k) \left[ G_{\omega k}^0 \right] \equiv \frac{5}{d} \int \frac{d^d k}{(2\pi)^d} \left[ G_{\omega k}^0 \right]^2 + 4\beta_d \left( \frac{d k}{d^2} \right)^2 \left[ G_{\omega k}^0 \right] \tag{A.9}
\end{equation}
originating from expanding the self-energy part up to \( q^4 \), gives a contribution to the scaling of the 4th-order moment that is of the same order of magnitude as the second term in Eq. (A.3). The angular average
\begin{equation}
\beta_d \equiv \langle \cos^4 \theta \rangle = \frac{\int_0^\pi \int_0^\pi \sin^2 \theta \sin^2 \theta \cos^4 \theta}{\int_0^\pi \int_0^\pi \sin^2 \theta \sin^2 \theta} = \frac{3}{d(d + 2)} \tag{A.10}
\end{equation}
in the spherical coordinates in \( d \) dimensions, entering the last term of Eq. (A.9), can be expressed using the spherical volume element by reducing the integrals to Euler’s \( B \)-functions.

\[ \Sigma_{d}(\omega) \]
\[ \text{with } A \text{ given by Eq. (A.6). We can see that, e.g., for the short-range disorder in any dimension, } p = 0, \text{ the contribution } \Sigma_d(\omega) \text{ vanishes, but in general it does not — e.g., for the case of } p = -1 \text{ in } d = 3 \text{ considered in Node 2.2.1.2 of Fig. 1.} \]

We are now ready to calculate \( \langle x^4 \rangle \) by substituting Eqs. (A.12) and (A.7) into Eq. (A.3). We perform the integration in the complex plane of \( \omega \) by rotating the path of integration to pass along the two sides of the branch cut of \( \omega^\theta \) which is convenient to choose along the negative imaginary axis. In this way, we obtain
\begin{equation}
\langle x^4 \rangle = 12 D_0^2 t^2 + 24 \left( 2 \cdot p(3 p + d - 4) \right) \cdot \frac{AD_0 r^{2-\theta}}{2(d + 2)} \tag{A.13}
\end{equation}
The leading term of \( \langle x^2 \rangle^2 \) (to the order \( O(\delta D^2) \sim O(A) \)), using Eq. (A.8), reads
\begin{equation}
\langle x^4 \rangle \approx 4D_0^2 t^2 + 8C_D D_0 r^{2-\theta} \tag{A.14}
\end{equation}
Finally, using the definition \( K(t) \equiv \langle x^4 \rangle / \langle x^2 \rangle^2 - 3 \), and again keeping only the lowest-order terms in \( C_D \sim A \) (cf. Eq. (8)), we obtain our main analytical result — Eq. (10) with
\begin{equation}
c_K = \frac{6C_D}{D_0} \left( 2 + \frac{p(3 p + d - 4)}{2(d + 2)} \right) \cdot \frac{1}{2 - \theta} - 1 \tag{A.15}
\end{equation}
yielding the ratio (11) in the main text. While the scaling with \( A/D_\infty \) of the result (A.15) could be guessed from the dimensional considerations, the dependence on \( p \) and \( d \) is nontrivial. Remarkably, due to the term in \( \Sigma_4 \), the tail in kurtosis depends separately on \( p \) and \( d \), rather than on the exponent (5) alone. Therefore, measuring the tails in both \( D(t) \) and \( K(t) \) can allow one to determine the structural exponent \( p \) and the effective dimensionality \( d \) separately, whereas measuring just the diffusion coefficient only yields their sum.

While obtaining Eq. (13) from Eq. (A.15) for \( p = 0 \) and \( d = 1 \) is straightforward, the \( \theta = 1 \) case is formally singular. To resolve this singularity in Eq. (A.15), we take an \( \epsilon \to 0^- \) limit of \( \theta = 1 + \epsilon \) in Eq. (10). For instance, for \( p = 0 \) and \( d = 2 \),
\begin{equation}
K(t) \approx 6A \ln t - \frac{1}{6} \frac{1}{D_\infty} t^{1-\epsilon} \tag{A.16}
\end{equation}
To better understand the physical meaning of the regularizer \( 1/\epsilon \) (reminiscent of the dimensional regularization in quantum field theory), we explore a similar singularity in the dispersive diffusivity in Eq. (A.7):
\begin{equation}
D_\omega \approx D_\infty + A \cdot i \omega \ln(-i \omega) + \frac{1}{\epsilon} \tag{A.17}
\end{equation}
where we used the Laurent expansion of the Euler’s \( \Gamma \)-function
\[ \Gamma_E(1 - \epsilon) \approx \frac{1}{\epsilon} \text{ and } (-i \omega)^p \approx 1 + \epsilon \ln(-i \omega) \] to simplify this formula. The singularity in Eq. (A.17) originates from the time
scale \sim \delta t$, from which the power-law tail begins. Burcaw et al. (2015) showed that, for $p = 0$ and $d = 2$, the dispersive diffusivity is given by

$$D(\omega) \approx D_0 + A \cdot \omega \ln(-i \omega \delta t).$$  \hspace{1cm} \text{(A.18)}$$

Comparing Eqs. (A.17) and (A.18), we identify $\frac{1}{12}$ with $\ln \delta t$, which after substituting into Eq. (A.16) yields Eq. (14).

Similar considerations yield Eq. (15), albeit with a coefficient that has a nontrivial contribution from $\Sigma$ due to nonzero $p$.

We note that the reason for the singularities at $\vartheta = 1$ is the fact that we measure the cumulative, rather than the instantaneous diffusion coefficient, for which the integral in Eq. (7) becomes insensitive to the tails decreasing faster than $1/t$. This is a feature of our PGSE measurement, rather than of the underlying physics of diffusion. Similar considerations apply to the (cumulative) kurtosis. Had we worked with the instantaneous 2nd and 4th order cumulants, e.g., defining them via $\delta_t \langle \chi^4(t) \rangle$, such a problem would not have arisen. The tail ratio (11) therefore can be generalized onto the power-law tails of the suitably defined instantaneous diffusivity and kurtosis for any $\vartheta$.

Appendix B. Accurate simulation of membrane permeability for finite-step MC simulations

Appendix B.1. The physics of the permeability correction. Equal molecular concentrations

The permeation probability $P$ through a membrane of permeability $\kappa$ depends on the distance $\delta s$ between the random walker and the encountered membrane when the distance is smaller than the step size $\delta x = \sqrt{2 D \delta t}$, with $D_0$ the intrinsic diffusivity and $\delta t$ the time-step in $d$ dimensional space, as derived in Appendix A of (Fieremans et al., 2010), Eq. (43):

$$P \equiv P_0 \frac{2 \delta s}{D_0},$$  \hspace{1cm} \text{(B.1)}

The functional form of $P$ is well-regularized even for the highly permeable membrane: the limit $\kappa \to \infty$ yields probability $P \to 1$, as expected.

However, calculating the distance from random walkers to encountered membranes can be slow in actual implementations, especially for simulations using complicated shapes. To simplify simulations, we would like to approximate $\delta s$ with $\delta x$, by averaging over possible step sizes $\delta s$ (up to $\delta x$), and introducing a constant $C_d$ to account for this approximation in $d$ dimensions. For that, let us first assume low probability ($P \ll 1$), such that the denominator in the left-hand side of Eq. (B.1) can be neglected. This yields the permeation probability

$$P \approx \kappa_0 \delta x \cdot C_d,$$  \hspace{1cm} \text{(B.2)}

where $\kappa_0$ is the input permeability value (whose difference from the genuine $\kappa$ will be explained below), and $C_d = 1, \pi/4$, and 2/3 for $d = 1, 2,$ and 3 (Powles et al., 1992; Szafer et al., 1995; Fieremans and Lee, 2018). Eq. (B.2) is applicable when the assumption $P \ll 1$ is satisfied, i.e.

$$\kappa_0 \ll \sqrt{\frac{D_0}{2d\delta t}} \cdot \frac{1}{C_d},$$

indicating that, for a large $\kappa_0$, a sufficiently small time step $\delta t$ is applied; in this case, $\kappa \approx \kappa_0$.

Let us now extend this approximation of $\delta s$ by $\delta x$ onto large $\kappa$, for which the input $\kappa_0$ would be significantly different from $\kappa$. It turns out that averaging over $\delta s$ simply renormalizes the input $\kappa_0$ entering Eq. (B.2), to achieve a genuine $\kappa$, Eq. (B.8) below. To derive this result, one needs to realize that averaging over $\delta s$ influences not only the permeation probability but also the calculation of particle flux density $j$. To solve this problem, we demand the Fick’s first law to be satisfied with the permeability $\kappa_0$ to be achieved in simulations, is related to the particle density difference at the membrane, i.e. $\phi_{10} - \phi_{20}$. As a result, the genuine permeability $\kappa$ is always larger than the input value $\kappa_0$, cf. Eq. (B.8).

Considering a permeable membrane at position $x = 0$ with an input $\kappa_0$ for the calculation of $P$, the intrinsic diffusivity over the left and right sides of the membrane is $D_1$ and $D_2$ respectively (Fig. B.1). The particle flux density from left to right (1 \to 2) is given by

$$j_{1 \to 2} \approx \frac{\langle \phi_1 \rangle_1 \cdot (S \delta x_1) \cdot P_{1 \to 2}}{S \cdot \delta t},$$  \hspace{1cm} \text{(B.3)}

where $S$ is the surface area, $P_{1 \to 2}$ is the permeation probability from left to right given by Eq. (B.2), and $\langle \phi_1 \rangle_1$ is the particle density averaged over the layer on the left side of the membrane, of thickness $\delta x_1$, with the consideration of hopping orientation $\Omega$ along which particles encounter the membrane.

Substituting Eq. (B.2) into Eq. (B.3) and using $(\delta x_1)^2 / \delta t = 2d D_1$, we obtain $j_{1 \to 2} \approx \kappa_0 \cdot (\langle \phi_1 \rangle_1 \cdot 2dC_d$. Similarly, the particle flux density from right to left side is $j_{2 \to 1} \approx -\kappa_0 \cdot (\langle \phi_2 \rangle_2 \cdot 2dC_d$. Then the net particle flux density is given by

$$j = j_{1 \to 2} + j_{2 \to 1} \approx \kappa_0 \cdot ((\langle \phi_1 \rangle_1 - \langle \phi_2 \rangle_2) \cdot 2dC_d.$$  \hspace{1cm} \text{(B.4)}
Given that the particle density right at left and right surface of the membrane is \( \phi_{01} \) and \( \phi_{02} \) (without spatial averaging), the net particle density flux is (by definition of the genuine \( \kappa \))

\[
\begin{align*}
  j &= \kappa \cdot (\phi_{01} - \phi_{02}) \\
  &= -D_1 \cdot \partial_x \phi_{01} - D_2 \cdot \partial_x \phi_{02}
\end{align*}
\]  
(B.5)

where \( \kappa \) is the genuine permeability we would like to achieve with simulations, different from the input value \( \kappa_0 \) and \( \partial_x \phi_{01} \) and \( \partial_x \phi_{02} \) are density gradients right at left and right surface of the membrane.

Here we average the density \( \langle \phi_1 \rangle \Omega \) (\( \langle \phi_2 \rangle \Omega \)) over the layer on the left (right) side of the membrane, of thickness \( \delta x_1 \) (\( \delta x_2 \)), and equate the flux density in Eq. (B.4) to that in Eq. (B.5) to obtain the genuine permeability \( \kappa \).

Approximating the particle density \( \langle \phi_1, \phi_2 \rangle \) variation close to the membrane with a linear function of the distance from the membrane, we have

\[
\begin{align*}
  \phi_1(x) &= \phi_{01} + \partial_x \phi_{01} \cdot x, \\
  \phi_2(x) &= \phi_{02} + \partial_x \phi_{02} \cdot x.
\end{align*}
\]

Then the particle density averaged within the thickness of step size (\( \delta x_1 \) and \( \delta x_2 \)), considering the hopping orientation \( \Omega \) along which particles encounter the membrane (Fig. A.1 in (Fieremans and Lee, 2018)), is

\[
\begin{align*}
  \langle \phi_1 \rangle \Omega &= \int_{-\delta x_1}^{0} \phi_1(x) \frac{\Omega(x) dx}{\delta x_1} = \frac{1}{2dC_d} \left( \phi_{01} - \partial_x \phi_{01} \cdot \delta x_1 \cdot \frac{C_d}{2} \right), \\
  \langle \phi_2 \rangle \Omega &= \int_{0}^{\delta x_2} \phi_2(x) \frac{\Omega(x) dx}{\delta x_2} = \frac{1}{2dC_d} \left( \phi_{02} + \partial_x \phi_{02} \cdot \delta x_2 \cdot \frac{C_d}{2} \right),
\end{align*}
\]  
(B.7a)

where

\[
\Omega(x) = \begin{cases} 
  \frac{1}{2} \cos^{-1} \left( \frac{x}{L} \right) / \pi & d = 1, \\
  \left( 1 - \frac{x}{L} \right)^{1/2} & d = 2, \\
  \left( 1 - \frac{x}{L} \right)^1 & d = 3.
\end{cases}
\]

Substituting Eqs. (B.4) and (B.6)–(B.7) into Eq. (B.5) yields

\[
\kappa = \frac{k_0}{1 - \alpha},
\]

(B.8)

where

\[
\alpha = \frac{1}{2} \left( \frac{\delta x_1}{D_1} + \frac{\delta x_2}{D_2} \right) C_d
\]

(B.9)

For example, in Section 3.5, we applied \( \Delta t = 0.002 \) ms, \( D_1 = D_2 = 2 \mu m^2/\mu s \), and \( k_0 = 0.4154 \mu m/\mu s \) in 1d simulations, yielding \( \alpha = 0.019 \) and a 2\% correction to the actual permeability \( \kappa = 0.4233 \mu m/\mu s \).

Interestingly, the correction factor \( \alpha \) is the permeation probability averaged for both directions, i.e. \( \alpha \in [0,1] \). Therefore, the genuine permeability \( \kappa \) in Monte Carlo simulations of any dimension \( d \) is always larger than the input value \( \kappa_0 \), as in Eq. (B.8), where the correction factor \( \alpha \) is essential especially when simulating the diffusion across a highly permeable membrane. To minimize \( \alpha \) and reduce the bias, a smaller time-step and larger intrinsic diffusivity should be used.

Furthermore, the corrected permeation probability, obtained by substituting Eq. (B.8) into Eq. (B.2), should still be \( \ll 1 \), leading to the following constraint, as a guidance of choosing simulation parameters:

\[
\kappa \ll \sqrt{\frac{2}{d \Delta t} \cdot \frac{1}{C_d} \cdot \frac{\sqrt{D_1 D_2}}{\sqrt{|D_1 - D_2|}}}.
\]

In other words, Eq. (B.8) works particularly well for a small time-step, large intrinsic diffusivities, and similar intrinsic diffusivities between compartments (\( D_1 = D_2 \)).

Practically, to simulate a membrane of permeability \( \kappa \), we have to tune the input permeability \( \kappa_0 \) for the permeation probability in Eq. (B.2) based on

\[
\kappa_0 = \frac{\kappa}{1 + \kappa \cdot (\alpha/k_0)},
\]

where the right-hand side is independent of \( \kappa_0 \) due to Eq. (B.9).

The above correction ensures the genuine permeability \( \kappa \) in simulations.

**Appendix B.2. General case: different spin concentration at both sides of the membrane**

In the previous section, the medium is assumed to have the same spin concentration in all compartments. However, a lower spin concentration is expected for some tissue microstructure, such as myelin water. To generalize for different spin concentrations in each compartment, the permeation probability in Eq. (B.2) is re-written as

\[
\begin{align*}
  P_{1\rightarrow 2} &= \frac{\kappa_0 \delta x_1}{D_1} \cdot C_d \cdot \left( \frac{C_2}{C_1} \right)^{\lambda}, \\
  P_{2\rightarrow 1} &= \frac{\kappa_0 \delta x_2}{D_2} \cdot C_d \cdot \left( \frac{C_2}{C_1} \right)^{\lambda-1},
\end{align*}
\]  
(B.10a)

(B.10b)

where \( C_1 \) and \( C_2 \) are spin concentrations over the left and right compartments of the membrane, and \( \lambda \in [0,1] \) is an exponent determined later. It is worthwhile to notice that the probability ratio \( P_{1\rightarrow 2}/P_{2\rightarrow 1} = C_2 \sqrt{D_2}/C_1 \sqrt{D_1} \) is maintained to ensure the particle density equilibrium for all diffusion times.

Similar to the derivation in previous section, substituting Eq. (B.10) into Eq. (B.3) and calculating \( j_{1\rightarrow 2} \) and \( j_{2\rightarrow 1} \), we obtain

\[
\begin{align*}
  j &= j_{1\rightarrow 2} + j_{2\rightarrow 1} \\
  &= \kappa_0 \left[ \langle \phi_1 \rangle \Omega \cdot \left( \frac{C_2}{C_1} \right)^{\lambda} - \langle \phi_2 \rangle \Omega \cdot \left( \frac{C_2}{C_1} \right)^{\lambda-1} \right] \cdot 2dC_d.
\end{align*}
\]  
(B.11)

Considering the ratio \( C_2/C_1 \) of spin concentrations, the net particle flux density is given by

\[
\begin{align*}
  j &= \kappa \left[ \phi_{01} \cdot \left( \frac{C_2}{C_1} \right)^{\lambda} - \phi_{02} \cdot \left( \frac{C_2}{C_1} \right)^{\lambda-1} \right] \\
  &= -D_1 \partial_x \phi_{01} = -D_2 \partial_x \phi_{02}.
\end{align*}
\]  
(B.12)

(B.13)
where the unbiased permeability $\kappa$ is re-defined accordingly.

Substituting Eqs. (B.7), (B.11) and (B.13) into Eq. (B.12) yields

$$\kappa = \frac{\kappa_0}{1 - \alpha_1}, \quad (B.14)$$

where

$$\alpha_1 = \frac{1}{2} \frac{\delta x_1}{D_1} \left( C_2 \frac{C_1}{C_1} + \frac{\delta x_2}{D_2} \left( \frac{C_2}{C_1} \right)^{1-1} \right) \cdot C_d,$$

The derivation of this extra permeability is similar to those in previous sections. Substituting Eqs. (B.7), (B.16a) and (B.16b) into Eq. (B.3), the particle density transition over the membrane is given by

$$j_{1 \rightarrow 2} \approx \kappa_0 \left( \phi_{0,1} - \phi_{0,1} \cdot \frac{C_d}{2} \right) \cdot \frac{C_2}{C_1},$$

$$j_{2 \rightarrow 1} \approx -\kappa_0 \left( \phi_{0,2} + \phi_{0,2} \cdot \frac{C_d}{2} \right) \cdot \frac{C_2}{C_1},$$

where

$$\kappa_0 = \sqrt{\frac{D_2}{2d \cdot \delta l}} \cdot \frac{1}{C_d}.$$
Zhang, Y., Brady, M., Smith, S., 2001. Segmentation of brain MR images through a hidden markov random field model and the expectation-maximization algorithm. IEEE transactions on medical imaging 20 (1), 45–57.
Figure S.1: Ice-water phantom diffusivity and kurtosis fitted values with respect to diffusion time at temperature = 0°C. No time-dependence was observed as expected, eliminating possible pulse sequence contributions to the time-dependence observed in cortical gray matter.

Figure S.2: Histograms of the mean kurtosis for each subject and time-point studied in this work. The histogram suggests reasonable inter-subject variability.