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High-Resolution Models of Motion of Macromolecules in Cell Membranes

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Abstract

The path of a macromolecule on a cell membrane is modeled by a sum of independent identically distributed random variables. Random variables with simple discrete distribution functions capture some important aspects of the jump or hop diffusion reported from single particle tracking experiments that measure the motion of single molecules on a cell membrane. The detail provided by the distribution function for the random variables is critical for accurate simulations of the motion and interactions of macromolecules on the cell membrane. Additionally, the probability distribution for the random variables is easily estimated from single-particle tracking data. The diffusion constant is given by the second moment of the probability distribution, which agrees with the diffusion constant estimated from the mean-square displacement, and thus represents far less information than the distribution function.

Keywords

cell membrane; macromolecules; anomalous diffusion; hop diffusion; jump diffusion; random walks; random variables

1 Introduction

The goal is to provide a step toward the detailed modeling of the motion, and especially the interactions, of macromolecules on the cell membrane, which play an important role in signal transduction. Signal transduction events can be controlled by a few macromolecules localized to a small area of the cell membrane. This precludes the use of continuum models for understanding these events. Substantial biological data points to the motion of macromolecules being anomalous, non Brownian, hop, jump, confined and sub-diffusion [1–12], which precludes the use of standard stochastic models based on simple Brownian motion. In the past, this motion was characterized by a diffusion constant or a diffusion constant combined with an anomalous diffusion exponent or a directed motion speed [12], and sometimes using models with several more parameters. To better account for the complexity of the motion, the diffusion constant has been considered time dependent or given by a distribution of values [10,13]. A diffusion constant represents information averaged over large times and many molecules. Even if several parameters are used, this averaging removes most of the information needed for detailed modeling over small spatial distances and short times.

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The detailed motion of macromolecules on the cell surface is commonly studied using single-particle tracking (SPT) [3,4,14], which produces a list of coordinates of a particle at an evenly spaced times. In the literature, the data from the SPT experiments are analyzed by fitting the MSD of the macromolecules with simple functions containing the parameters mentioned above and occasionally by using the cumulative distribution (CDF) of the distance traveled over some period of time [6,12,15]. Additional recent results on SPT are given in [7,9,16–22]. Some explanations of the anomalous diffusion depend on confinement by cytoskeletal fences [14, 4], and the motion has been modeled as a confined Brownian diffusion, but [1,23] cast some doubt on this interpretation. The article [24] discusses the limitation on the spatial accuracy attainable in single-molecule microscopy. Because photobleaching experiments involve a large number of paths over a large time, it may be possible to model these experiments using a diffusion constant and Brownian motion, but there are problems even in this case [18,25,10]. Also, total internal reflection fluorescence microscopy [26], can be used to image single molecules inside a cell, and wide-field fluorescence microscopy can be used to track particles near, not just on, the cell membrane [27]. Recently, multiple particle tracking measurements in volume have been used to understand the properties of networks of actin [28,29]. Multiple particles on living cells can also be tracked using quantum dots [30–32].

From SPT data it is easy to estimate the CDF of the jump sizes and angles between jumps. These probability densities provide the detail required for the modeling of the chemistry of the macromolecules at low density. Thus our modeling will focus on the probability distribution functions (PDFs) rather than the diffusion constant. It is expected that the jumps are uniform in the angles (which seems not to have been checked for data in the literature), so that the important information is only in the PDF for the jumps. We find that important aspects of membrane diffusion can be modeled using a discrete radial PDF with two jump lengths. Because of the complexity of the living cell membrane, it is not expected that diffusion will completely explain the motion of macromolecules.

Given the PDF for the jump sizes, the expected displacement is easily shown to be zero and the second moment of the random variable is given by an integral the radial probability function. The diffusion constant for the random walk is proportional to this second moment and is consistent with the value obtained from the mean square displacement. The Central Limit Theorem says that the average of the sum of a large number of independent, identically distributed random variables with finite means and variances converges to a normal random variable. There are many precise variants of this result, e.g. Donsker's Theorem. So our models will produce Brownian motion on large time scales.

Our model uses uniformly distributed angles and a discrete radial distribution with two jump lengths deduced from the live cell data given on the Kusumi web page [33] and produces paths that are similar to the Kusumi data. For artificial membranes [33], the simulated paths are even more like the data. Interestingly, the MSD for simulated paths is erratic, even when averaged over a large number of paths. Most of our modeling is done in the plane, but we do present some simulations on a sphere approximately the same size as the cells used in [33].

Additionally, the tools developed are useful in analyzing data. If the diffusion is Brownian, then the distribution of the coordinates should be symmetric and normal which is equivalent to the angles being uniformly distributed and the radial distribution being Weibul. The analysis begins by checking this. We are currently working on applying time series analysis tools to SPT data. We thank M.C. Zúñiga for generously sharing her research group's primary data [3] to test these ideas. An important result here is that for some of the data, the jumps are correlated.

There is no attempt to explain the underlying physical causes of anomalous diffusion, but the results given in [5] indicate that the confinement is due to an effective harmonic potential generated by long-range attraction between the membrane proteins. We have a project underway [34] to also include such interactions.

Section 2 reviews some basic results about random walks. Section 3 gives a method to create a random variable that models SPT data. Section 4 uses the derived Markov-chain Monte-Carlo algorithms to simulate some data given on the Kusumi Laboratory web page [33]. Section 5 uses the developed model to analyze some data from [3]. Section 6 extends the simulations to the surface of sphere that is approximately the size of the cells used in SPT studies. Section 7 gives a summary, conclusions, and future directions. In this paper, distance is measured in nanometers (nm) and time in microseconds (μ s). Consequently, diffusion constants are given in $nm^2/\mu s = \mu m^2/s = 10^{-8}cm^2/s$.

2 Random Walks In 2-D

A small patch of the cell membrane can be modeled as a plane and a step in a random walk of a macromolecule in the membrane can modeled using a random variable J (for jump). The plane has Cartesian coordinates (x, y) and polar coordinates (r, θ) with $x = r \cos(\theta)$ and $y = r \sin(\theta)$. The random variable

$$\vec{J}=(x,y)=(r \cos(\theta), r \sin(\theta)) \quad (1)$$

is determined by a probability density $J(r, \theta) \geq 0$ with

$$\int_0^\infty \int_0^{2\pi} J(r, \theta) dr d\theta = 1. \quad (2)$$

It will be assumed that the motion in r and θ are independent, so

$$J(r, \theta) = R(r) \Theta(\theta), \quad \int_0^\infty R(r) dr = 1, \quad \int_0^{2\pi} \Theta(\theta) d\theta = 1. \quad (3)$$

If the probability of a jump is independent of direction, then $\Theta(\theta) = 1/(2\pi)$. In the case of a discrete probability density,

$$R(r) = \sum_{k=1}^K p_k \delta(r - r_k), \quad (4)$$

where $0 < K \leq \infty$, δ is the Dirac delta function, $p_j > 0$, $r_j \geq 0$ and

$$\int_0^\infty R(r) dr = \sum_{k=1}^K p_k = 1. \quad (5)$$

With $N > 0$, let $\vec{J}_i$, $1 \leq i \leq N$, be independent identically distributed (IID) random variables with probability density

$$J_i(r, \theta) = \frac{R(r)}{2\pi}. \quad (6)$$

Then the expected value of $\vec{J}_i$ is

$$\langle \vec{J}_i \rangle = \int_0^{2\pi} \int_0^\infty (r \cos(\theta), r \sin(\theta)) \frac{R(r)}{2\pi} dr d\theta = (0, 0), \quad (7)$$

the expected value of $\vec{J}_i \cdot \vec{J}_i = \|\vec{J}_i\|^2$ is

$$\begin{aligned}\langle \vec{J}_i \cdot \vec{J}_i \rangle &= \int_0^{2\pi} \int_0^\infty (r \cos(\theta), r \sin(\theta)) \cdot (r \cos(\theta), r \sin(\theta)) \frac{R(r)}{2\pi} dr d\theta \\ &= \int_0^\infty r^2 R(r) dr = \langle r^2 \rangle,\end{aligned}\quad (8)$$

and the expected value of $\vec{J}_i \cdot \vec{J}_j$ with $i \neq j$ is

$$\langle \vec{J}_i \cdot \vec{J}_j \rangle = \langle \vec{J}_i \rangle \cdot \langle \vec{J}_j \rangle = (0,0) \cdot (0,0) = 0. \quad (9)$$

In the discrete case,

$$\langle r^2 \rangle = \int_0^\infty r^2 \sum_{k=1}^K \delta(r - r_k) p_k dr = \sum_{k=1}^K r_k^2 p_k. \quad (10)$$

The random motion of a macromolecule starting the origin is modeled by

$$\vec{P}_N = \sum_{i=1}^N \vec{J}_i. \quad (11)$$

The expected value of the position of a macromolecule is

$$\langle \vec{P}_N \rangle = \langle \sum_{i=1}^N \vec{J}_i \rangle = \sum_{i=1}^N \langle \vec{J}_i \rangle = (0,0). \quad (12)$$

The expected value of the square displacement of a macromolecule is

$$\langle \vec{P}_N \cdot \vec{P}_N \rangle = \langle \sum_{i=1}^N \vec{J}_i \cdot \sum_{j=1}^N \vec{J}_j \rangle = \sum_{i=1}^N \sum_{j=1}^N \langle \vec{J}_i \cdot \vec{J}_j \rangle = \sum_{i=1}^N \langle \vec{J}_i \cdot \vec{J}_i \rangle = N \langle r^2 \rangle. \quad (13)$$

In two dimensions, the diffusion equation for $f(x, y, t)$ with diffusion constant D and its Green's G function are

$$\frac{\partial f}{\partial t} = D \left(\frac{\partial^2 f}{\partial x^2} + \frac{\partial^2 f}{\partial y^2} \right), \quad G(r, t) = \frac{1}{2} \frac{1}{D} e^{-\frac{r^2}{4Dt}}. \quad (14)$$

Also, G is positive and has integral one, and thus $p(r, t) = rG(r, t)$ is a probability distribution (called the Weibull distribution) with cumulative distribution function

$$C(r, t) = 1 - e^{-\frac{r^2}{4Dt}}. \quad (15)$$

The expected value of the square displacement is given by

$$E(r^2, rG) = \int_0^\infty r^3 G(r, \theta, t) dr d\theta = 4Dt. \quad (16)$$

To connect a random walk with N steps to the diffusion equation, choose $\Delta t > 0$ and then set $t = N \Delta t$. Comparing (13) and (16) suggest defining the diffusion constant for any random walk as

$$D = \frac{1}{4t} \langle \vec{P}_N \cdot \vec{P}_N \rangle = \frac{\langle r^2 \rangle}{4\Delta t}. \quad (17)$$

This definition makes sense for any random walk with a probability density with a finite second moment. As noted in the introduction, the long-time limit of a random walk with finite second moment will converge to Brownian motion with a diffusion constant given by (17).

In the biophysics literature, diffusion coefficients in single-particle tracking are estimated using the mean square displacement (MSD) which depends on a number of steps n . Let $\vec{r}_i = (x_i, y_i)$, $1 \leq i \leq N$ be a particle path and $\|\vec{r}_i\|^2 = x_i^2 + y_i^2$. Then

$$\text{MSD}_n = \frac{1}{N-1-n} \sum_{i=1}^{N-1-n} \|\vec{r}_{i+n} - \vec{r}_i\|^2. \quad (18)$$

Only small values of n ($n < N/10$) are used. If the paths are generated by a random variable with probability density given by (6), then the expected value of MSD is

$$\langle \text{MSD}_n \rangle = \frac{1}{N-1-n} \sum_{i=1}^{N-1-n} n \langle r^2 \rangle = n \langle r^2 \rangle. \quad (19)$$

So the diffusion constant can be estimated by (17):

$$D = \frac{\langle r^2 \rangle}{4 \Delta t} = \frac{\langle \text{MSD}_n \rangle}{4 n \Delta t} = \frac{\langle \text{MSD}_n \rangle}{4 t}, \quad (20)$$

which is the standard formula given in the literature written in our notation. As the expected value of the MSD is a straight line, the diffusion constant can be computed from the slope of the straight line. Approximating the derivative by a difference quotient gives $D = (\text{MSD}_{n+1} - \text{MSD}_n)/4\Delta t$, which is also used to estimate the diffusion constant (see the *Gold Particle Tracking and Data Analysis* section of [3]).

2.1 Anomalous Diffusion

Mathematically, from the above analysis it is clear that if a general random walk is given by IID random variables J_i whose PDFs have finite second moments, then the diffusion is *normal* with diffusion constant proportional to the average of the second moments of the PDFs (see formula (13)). However, as will be seen, if the PDFs are not simple, then such random walks can produce paths that appear anomalous over short time periods. Thus, mathematically, anomalous diffusion can only occur if one of the IID or finite second moment assumptions are violated.

If the random variables are not IID, specifically, if the random variables are not independent, then formula (13) becomes

$$\langle \vec{P}_N \cdot \vec{P}_N \rangle = \left\langle \sum_{i=1}^N J_i \cdot \sum_{j=1}^N J_j \right\rangle = \sum_{i=1}^N \sum_{j=1}^N \langle J_i \cdot J_j \rangle. \quad (21)$$

The most extreme case of correlated random variables is when the particle moves a given distance d in a given direction each time step, for example, when a particle is moving in a constant velocity field without any random motion. In this case,

$$J_i \cdot J_j = d^2 \quad \text{and} \quad \langle \vec{P}_N \cdot \vec{P}_N \rangle = N^2 d^2. \quad \text{Then (18) gives} \\ \text{MSD}_n = n^2 d^2 = t^2 v^2, \quad v = \frac{d}{\Delta t}, \quad (22)$$

that is, the MSD is quadratic in time rather than linear. Although the notion of a diffusion constant depends on the independence of the random variable, and thus doesn't apply in this case, formula (22) gives that the diffusion constant isn't constant, but linear in time. Another possibility is to drop the assumption that the second moment of the random variables are finite, which leads to modeling using Lévy flights and fractional-order diffusion equations [35–38].

3 SPT Data

SPT data can be analyzed by computing the cumulative distribution function for the size and angles of the jump. Let $\vec{r}_i = (x_i, y_i)$, $1 \leq i \leq N$, be a particle path and

$$\vec{\Delta r}_i = \vec{r}_i - \vec{r}_{i-1}, 2 \leq i \leq N, \text{ and then let } d_i = \|\vec{\Delta r}_i\| = \sqrt{\Delta x_i^2 + \Delta y_i^2}.$$

Next, for $2 \leq i \leq N$, let

$$X_i = \frac{\vec{\Delta r}_{i-1} \cdot \vec{\Delta r}_i}{\|\vec{\Delta r}_{i-1}\| \|\vec{\Delta r}_i\|}, \quad Y_i = \vec{k} \times \frac{\vec{\Delta r}_{i-1} \cdot \vec{\Delta r}_i}{\|\vec{\Delta r}_{i-1}\| \|\vec{\Delta r}_i\|}, \quad \theta_i = \text{Atan}(X_i, Y_i), \quad (23)$$

where $\text{Atan}(X, Y)$ returns an angle θ with $-\pi < \theta \leq \pi$, $\cos(\theta) = X / \sqrt{X^2 + Y^2}$ and $\sin(\theta) = Y / \sqrt{X^2 + Y^2}$. These formulas can be used to produce two sets of “random” numbers:

$$\cup_{i=2}^N \{d_i\} \quad \text{and} \quad \cup_{i=2}^N \{\theta_i\}.$$

If S is a finite set of random real numbers, then the set can be enumerated in increasing order: $S = \cup_{i=1}^I \{s_i\}$ where $s_{i+1} \geq s_i$. Then the most detailed estimate of cumulative distribution function for generating the set S is given by

$$\text{CDF}(s) = \begin{cases} 0 & s < s_1 \\ \frac{i}{I} & s_i \leq s < s_{i+1}, \quad 1 \leq i \leq I-1 \\ 1 & s_I \leq s \end{cases} \quad (24)$$

One can compute a less detailed estimate of the CDF more rapidly by binning the set S .

Once the the CDF for the jumps has been estimated, the probability distribution is the derivative of the CDF, $R(r) = \text{CDF}'(r)$. However, the second moment (8) of the probability distribution can be computed directly from the cumulative distribution. To estimate the second moment from a path, set $r_0 = 0$, and approximate the derivative by a difference quotient and the integral by the midpoint rule to give

$$\langle r^2 \rangle = \int_0^\infty r^2 R(r) dr \quad (25)$$

$$\approx \sum_{i=0}^{N-1} \left(\frac{r_{i+1} + r_i}{2} \right)^2 \frac{\text{CDF}(r_{i+1}) - \text{CDF}(r_i)}{r_{i+1} - r_i} (r_{i+1} - r_i) \quad (26)$$

$$= \frac{1}{N} \sum_{i=0}^{N-1} \left(\frac{r_{i+1} + r_i}{2} \right)^2. \quad (27)$$

Then the diffusion constant can be estimated by (17), $D = \langle r_2 \rangle / 4\Delta t$.

To test how close the CDF for the jumps is to a normal distribution, and consequently how close a path is to a Brownian motion, the CDF can be least-squares approximated by the Gaussian CDF (15).

4 Simulations

In slide 10 of the second slide show on the Kusumi Laboratory Web Page [33] there is a table of some single-particle tracking data for DOPE. These data are replicated in table 1, with some added data given by simple estimates. The Kusumi data is used because it is taken with a camera with a frame rate over a thousand times faster than the video-rate cameras typically used to

take data. A simple model for this jump diffusion is given by a probability distribution with two jump sizes,

$$p(r) = p_1 \delta(r - j_1) + p_2 \delta(r - j_2), \quad (28)$$

where j_1 is a small jump with large probability p_1 and j_2 is a large jump with small probability p_2 . The information in table 1 can be used to estimate a two-jump probability distribution. First assume that the jumps in a compartment

$$5.4 = D = \frac{\langle r^2 \rangle}{4 \Delta t} = \frac{j_1^2}{4 * 25}, \quad (29)$$

or $j_1 = 23\text{nm}$. As the compartments are almost touching, the large jump is chosen as the cluster diameter, $j_2 = 230\text{nm}$. The number of steps a macromolecule takes in a compartment is about 440 (see table 1), so the probability of a large jump is taken as $p_2 = 1/441$ and then $p_1 = 1 - p_2$. The second moment of this distribution gives $D_T = 6.4776$.

Before simulating jump diffusion, random motion in a single compartment is simulated using a probability distribution with a single jump $j = 23\text{nm}$ and $M = 100$ paths each with $N = 440$ steps ($M = 5$ paths are shown in figure 1). The theoretical diffusion constant given by (17) is 5.29, as is the estimate using the cumulative distribution. The estimate using MSD starts at 5.29 and slowly climbs with increasing n . These results agree with the value of 5.4 given by (29) (within the approximations made in the estimates used to calculate j). Additionally, the cumulative distribution for the angles is a straight line, indicating that the angles of the jumps are uniformly distributed. These results support the correctness of the codes. As expected, diffusion modeled by a single jump size appears more diffuse than the Kusumi Laboratory data.

Four simulations of jump diffusion are shown in figure 2. In these figures, the line color changes every time the path takes a large jump. These simulations certainly capture some of the features of the images in [33], but are also somewhat more random. It will take more than simple jump diffusion to model in detail single-particle tracking data. In slide show 3, slide 25, on [33] there are pictures of DOPE molecules diffusing in the membrane of a large unilamellar vesicle. These are strikingly like the simulations in figure 2.

4.1 The Central Limit Theorem

As noted before, simulations using the two jump probability distribution given above with M paths each containing N jumps, should converge to Brownian motion when N and M both become large. The expected distance traveled by a Brownian particle is proportional to $\sqrt{t} \propto \sqrt{N}$. For figure 3, $N = M = 25, 100, 400$ and the cumulative distribution functions are plotted against $r / \sqrt{N}$. Clearly these cumulative distributions are approaching a Gaussian. The diffusion constants in table 2 are computed by doing a least squares fit of cumulative distribution functions using (15). The estimates are still erratic using $N = M = 400$ or 160,000 steps, so the method of estimating D is not so good.

4.2 Comparing Estimates of Diffusion Constants

To compare the diffusion constant computed by using the estimated probability density and by using the MSD, we simulated M paths for each of $M = 1, 10, 100$ with each path containing $N = 1000$ jumps and the probability density given in (28), so that theoretically $D = D_T$. Two examples for each value of M are given. The MSD diffusion constant D is computed from (20) for single paths, then averaged over the M paths, and then plotted as a function of the time steps n in figure 4. The horizontal line is given by the diffusion constant computed from the second moment. The MSD diffusion constant is erratic beyond about ten time steps. However, for short times the diffusion constant is converging to the theoretical value as M becomes large.

To understand the accuracy of the diffusion constant D given by the second moment computed using numerical integration formula 25, in table 3, we give two estimates using $M = 1, 10, 100$ paths of $N = 1000$ steps. The error in the estimate is $\Delta D = D - D_T$. The accuracy of the estimates of the diffusion constant usually improves as M increases.

The main conclusion is that to analyze random paths that have built in jumps, the MSD is erratic. For one step ($n = 1$), the MSD estimate is similar to the second-moment estimate. The second-moment estimate is simpler and “cleaner” for the types of problems studied, and thus relevant to single-particle tracking data.

5 Analyzing Real Data

A research group [3] specializing in SPT has allowed us to use some of their data on the motion of class I major histocompatibility complexes on cell membranes to test our ideas. The paper [3] provides an extensive and detailed analysis of this motion including the computation of a microscopic diffusion based on the MSD for short times and the *relative deviation* of the MSD from a line to provide a quantitative classification of the motion. Our goal here is to use this data set to show our ideas are consistent with these methods.

The data we analyzed consisted of 34 probe tracks. This section presents the results of analyzing four “unusual” paths using the CDF and the MSD to compute the diffusion constant. We also tested to see if the angles between successive jumps were uniformly distributed. Detailed information about the data can be found in [3]. The coordinates of the positions are in nanometers and the time step is given by the standard video rate (1/30 second). The tracks for the four data sets are given in figure 5, figure 6, figure 7, and figure 8. Each of these figures contains four sub-figures: the first sub-figure gives the probe path; the second gives a horizontal line given by the diffusion constant obtained using the computed CDF and the MSD estimate of the diffusion constant (20); the third sub-figure gives a plot of the CDF of the jump lengths and a plot of the best mean square fit using (15); and the fourth sub-figure gives a plot of the CDF of the angles between the jumps and the line $(\theta + \pi)/2\pi$ which is the expected value of the CDF for uniformly distributed angles.

For all of the data sets, the plot of the diffusion constant estimated by the MSD starts near the line given by the CDF estimate of the diffusion constant indicating that our CDF estimate agrees with the micro diffusion constant in [3]. However, there was a wide range of types of paths, and consequently, a large variation in the behavior of the MSD estimates of the diffusion constant for n slightly bigger than 1. As predicted by the Central Limit Theorem the distribution of the jump sizes is closely fit by a Gaussian CDF (15). The diffusion constants (see table 4) computed from the second moment of the CDF of the jumps (17) and from the Gaussian fit are close and close to those given in figure 2A of [3]. Over all 34 data sets, the difference in the diffusion constants varied from .03 to $1.29 \cdot 10^{-2} \text{ nm}^2/\mu\text{s}$, that is, by nearly two orders of magnitude.

As discussed in the section 2.1, IID random walks whose PDF has a finite second moment cannot produce anomalous diffusion (in the mathematical sense). There are no apparent reasons for assuming that the data is not identically distributed (ID) nor to assume that the PDFs don't have a finite second moment. So to have anomalous diffusion the data would need to violate the the assumption of being independent (I). The lack of independence would require that (see equation (21)) $\langle J_i \cdot J_j \rangle \neq 0$ for some $i \neq j$. To create a measure of the independence we introduce

$$C_k = \frac{1}{N-k} \sum_{i=1}^{N-k} \langle J_i \cdot J_{i+k} \rangle. \quad (30)$$

Using a path $\vec{r}_i, 1 \leq i \leq N$, this measure can be estimated by

$$C_k = \frac{1}{N-k} \sum_{i=1}^{N-k} \vec{r}_i \cdot \vec{r}_{i+k} . \quad (31)$$

The C_k are the product of the two step sizes times the cosine of the angle between, so C_0 give an estimate of the average jump size squared while C_1 is closely related to the angles given in the plots. For $k > 0$, IID implies that $C_k = 0$. Consequently the estimates of these C_k should be significantly smaller than C_0 . To test this, we computed the estimate of C_k for four paths generated using our standard two jump probability distribution (29). For $k > 0$, C_k is less than 4% of C_0 . The C_k for each of the four experiments are presented in table 5. The values of C_1/C_0 range from 7 to 37%, so the jumps in the paths are significantly more correlated than the two-jump paths.

In figure 5 the path is localized to a small region approximately 400 by 500nm. The MSD diffusion constant decreases with time, indicating the probe is confined to a small area. The deviation from a straight line of the CDF for the angles indicates a correlation between the steps. The value of C_1/C_0 is 37% indicating strongly correlated steps. The CDF for the angles in the figure deviate significantly from a straight line.

In figure 6 the path is also localized to a region of approximately 3000 by 700nm, but now there is a small “tail”. However, the estimate of the diffusion constant by the MSD now increases as function of the time. This could be due to the tail. The value of C_1/C_0 is 17% indicating correlated data. The CDF for the angles in the figure deviate significantly from a straight line.

In figure 7 the overall path has a linear displacement, but surprisingly the MSD estimate of diffusion constant decreases and stays low. The value of C_1/C_0 is 9% indicating slightly correlated data. The CDF for the angles gives a reasonable fit to a straight line.

In figure 8 the path now looks like it is traversing four confinement zones. This appears to be reflected in the MSD estimate of the diffusion constant by early underestimates of the diffusion constant. The value of C_1/C_0 is 7% indicating slightly correlated data. The CDF for the angles gives a reasonable fit to a straight line.

To classify sub-parts of the paths into different types of motion as done in [3] using the random variables will require a substantial extension of the theory presented here. We have recently started a new project to apply standard time series analysis to the jumps in the paths. So far our new results are consistent with the above, but the time series analysis is more precise.

6 Simulations on a Sphere

The goal of this section is to indicate how fast unhindered random motion given by the two jump probability distribution (29) can explore the surface of a sphere of radius $R = 7000\text{nm}$, which is approximately the size of a cell. The macromolecules on the cell membrane are modeled as points on the sphere. There are two problems: randomly distribute the initial points on the sphere for which there are several methods [39,40]; and then move the points a given distance in a random direction on the sphere. If a walker is at a point p on the unit sphere, we first find a point q the distance d , in a random direction, from the north pole. If the north pole is rotated to the point p , then the same rotation moves the point q to a point q' , the next point in the walk. This algorithm was implemented in MatLab (detailed formulas are given in the code) and used to produce the simulations shown in figure 9 and 10. These simulations used the two-jump probability distribution derived from the parameters in table 1, so they are the spherical analogs of the planar simulation shown in figure 2. We see that the diffusion rates

given in [33] produce rather rapid motion on the cell surface when there are no obstructions to the motion.

7 Summary

The use of IID random walks and probability distribution functions to model the motion of macromolecules on a cell membrane provides significantly more insight than does a diffusion constant (or even a generalized diffusion constant) because using a probability distribution can capture more details of the motion. Such motion can appear anomalous on short to modest time scales but, as the Central Limit Theorem implies, must look Brownian on at large times. A model of anomalous diffusion where the radial motion is given by two jump sizes was used to simulate the data in [33]. This simple model already produces single-particle paths like those on artificial membranes, and captures some aspects of the paths on cells. It is also important that these simulations indicate that, for this two-step distribution, the mean-square displacement behaves erratically on the simulated paths. Additionally, a simulator for the surface of sphere was developed to provide insight into the global motion of macro-molecules.

Data from [3] were used to study how well the PDF captured the details of the motion of the histocompatibility complexes on the cell membrane. The detailed methods of analysis used in [3] reveals that this motion is anomalous, while our analysis does not provide sufficient detail to draw this conclusion. It is important that the time step used in [3] is $4/3 \cdot 10^3$ times larger than the one used in [33] and our model is most appropriate for short times, and so is most applicable to the data with the shortest time step. Additionally, we discovered that there is some moderate but significant correlations between the jumps at different time steps in the data from [3]. This violates our IID assumption so we are now investigating the impact of correlations on random motion. It is clear that such correlations can produce diffusion that both appears anomalous and is anomalous in the mathematical sense. It is also important to analyze in more detail some high-speed data to see if anomalous diffusion can be detected at this highly resolved time scale.

We will also extend our methods to incorporate molecular interactions that will cause clustering of the macro-molecules and then study the impact of anomalous diffusion and clustering on the chemistry of signaling cascades. Given sufficient computing power, the spherical simulator can be used to study the impact of anomalous diffusion on photo-bleaching data. In the longer term, more realistic three-dimensional membrane structure will be included in the simulations by adding advanced version of the described algorithms to powerful simulation packages like the ChemCell simulator developed by Sandia Laboratories [41].

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Fig 1.
Sample simulated diffusion in a single compartment.

Fig. 2. Sample simulated two-jump diffusion. The paths consists of a sequence of short jumps all plotted in one color, separated by a large jump.

Fig 3.
The cumulative distribution function, plotted versus $r/\sqrt{N}$, showing the convergence to a Gaussian.

Fig. 4. Two estimates of the MSD diffusion constant D for $N = 1000$ and $M = 1$ in row 1; $M = 10$ in row 2; $M = 100$ in row 3. The red line is value of D estimated from the second moment of the jump sizes.

Fig. 5.
SPT path (Experiment Z4_001600_pt1_xy)

Fig 6.
SPT path (Experiment 221_00000000XptlX1_xy)

Fig 7.
SPT path (911_01183800_pt4_xy)

Fig. 8.
SPT path (Experiment Z1_003224_pt1_xy)

Random Motion of 100 Particle on a Sphere to Time 0.1 Seconds.

Fig. 9.
One hundred 1/10 of a second jump diffusion paths on a sphere.

Random Motion of 1 Particle on a Sphere to Time 1 Seconds.

Fig. 10.
A one-second jump diffusion path on a sphere.

Table 1

Data from the Kusumi laboratory for single-particle tracking for DOPE. are all of about the same size j_1 so that (see (10) and (17))

$\Delta t = 25 \mu s$	time step
$t = 62ms$	total time
$N = 2480$	number of time steps
$t = 11ms$	time in a compartment
$c = 6$	number of compartments
$n = 440$	number of time steps in a compartment
$r = 230nm$	compartment diameter
$D = 5.4 \mu m^2/s$	compartment diffusion constant (MSD)

Table 2

Four estimates of the diffusion constant $D_T = 6.4776$ when the number of steps N and the number of walks M increase.

N = M	1	2	3	4
25	7.116	6.624	7.190	6.290
100	5.922	6.423	6.887	6.726
400	6.888	5.988	6.766	6.738

Table 3

The diffusion constant given by the second moment of the computed probability density for two numerical experiments for $N = 1000$ and $M = 1, 10, 100$.

M	D	ΔD	D	ΔD
1	6.62	0.14	6.41	-0.07
10	6.61	0.13	6.84	0.36
100	6.45	-0.03	6.44	-0.04

Table 4

The CDF and Brownian fit diffusion constants ($10^{-2}\text{nm}^2/\mu\text{s} = 10^{-10}\text{cm}^2/\text{s}$).

figure	CDF	fit
5	1.8046	1.7105
6	0.1716	0.1463
7	1.7730	1.4489
8	2.9467	2.7086

Table 5

The correlation coefficients C_k for four of the experiments in [3]. N is the number of data points.

	N k	1131 A	1007 B	2018 C	912 D
	0	2416	230	2378	3951
	1	-891	-40	203	-296
	2	-189	-4	45	250
	3	62	8	-6	-65
	4	-195	-3	-23	316
	5	161	-2	-25	-18

A = Z4_001600_pt1_xy

B = 221_00000000XptlX1_xy

C = 911_01183800_pt4_xy

D = Z1_003224_pt1_xy