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Position dependent diffusion tensors in anisotropic media from simulation: oxygen transport in and through membranes

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Abstract

A Bayesian-based methodology is developed to estimate diffusion tensors from molecular dynamics simulations of permeants in anisotropic media, and is applied to oxygen in lipid bilayers. By a separation of variables in the Smoluchowski diffusion equation, the multidimensional diffusion is reduced to coupled one-dimensional diffusion problems that are treated by discretization. The resulting diffusivity profiles characterize the membrane transport dynamics as a function of the position across the membrane, discriminating between diffusion normal and parallel to the membrane. The methodology is first validated with neat water, neat hexadecane, and a hexadecane slab surrounded by water, the latter being a simple model for a lipid membrane. Next, a bilayer consisting of pure 1-palmitoyl 2-oleoyl phosphatidylcholine (POPC), and a bilayer mimicking the lipid composition of the inner mitochondrial membrane, including cardiolipin, are investigated. We analyze the detailed time evolution of oxygen molecules, in terms of both normal diffusion through and radial diffusion inside the membrane. Diffusion is fast in the more loosely packed interleaflet region, and anisotropic, with oxygen spreading more rapidly in the membrane plane than normal to it. Visualization of the propagator shows that oxygen enters the membrane rapidly, reaching its thermodynamically favored center in about 1 ns, despite the free energy barrier at the head group region. Oxygen transport is then quantified by computing the

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SUPPORTING INFORMATION AVAILABLE

The Supporting Information contains information on the force field, a visualization of the oxygen trajectories, the number of basis functions, the fitting of D from various lag times, a comparison of diffusivities and viscosities between simulations and experiment, and the particle and electron density of the inhomogeneous systems. This information is available free of charge via the Internet at <http://pubs.acs.org>. The code used to extract the diffusion and free energy profiles is freely available as the open-source software package **mcDiff** (<http://github.com/annekegh/mcDiff>).

oxygen permeability of the membranes and the average radial diffusivity, which confirm the anisotropy of the diffusion. The position dependent diffusion constants and free energies are used to construct compartmental models and test assumptions used in estimating permeability, including Overton's rule. In particular, a hexadecane slab surrounded by water is found to be a poor model of oxygen transport in membranes because the relevant energy barriers differ substantially.

Graphical Abstract

Keywords

lipid bilayers; inverse Monte Carlo; cardiolipin; POPC; Overton's rule; Bayesian analysis; Smoluchowski; anisotropy

I. INTRODUCTION

In the last steps of oxygen transport in the respiratory cycle, oxygen diffuses into the inner membrane of the mitochondria and gets trapped by cytochrome *c* oxidase (COX). The capture of oxygen by COX is a critical step in energy transduction by cells. As sketched in Fig. 1, oxygen enters the inner mitochondrial (or bacterial) membrane from the outside and diffuses to the oxygen binding site of COX located near the membrane center, between its two leaflets,^{1,2} where it is consumed to produce energy. Competing pathways include returning to the outside, and crossing the membrane into the interior of the mitochondrion (or bacterium). The probabilities of each path are determined by the position-dependent normal and radial components of the diffusion tensor. This paper develops the theory for extracting the full diffusion tensor in a planar layered system from molecular dynamics simulation.

The membrane is an inhomogeneous and anisotropic medium. Oxygen transport is thus described by an anisotropic diffusivity tensor that depends on the position in the membrane. Diffusion parallel to the bilayer surface in the radial direction ($\parallel$) differs from diffusion normal to the bilayer ($\perp$). Moreover, some regions are energetically more attractive for oxygen molecules than others, and the oxygen equilibrium distribution over the membrane follows a position-dependent free energy profile. Knowledge about these diffusion and free energy profiles will help us understand the pathway followed by oxygen to reach the presumed O₂ uptake channel of COX at the center of the bilayer, and of membrane permeability to oxygen.^{1,2}

The radial and normal diffusion profiles are not directly measured in experiment. However, molecular simulation provides explicit oxygen trajectories, which gives immediate access to the dynamics of oxygen transport. The inhomogeneity and anisotropy of the membrane still complicate the data analysis of the trajectories, since transport properties cannot be simply deduced by fitting a slope to the mean squared displacement (MSD) as function of time t , i.e., $MSD = 6Dt$, with D the diffusion coefficient. When this traditional procedure is applied in an inhomogeneous medium, the traveled distance reflects at longer times the net effects of regions with different free energies and diffusivities, e.g. the head-group region, the tail region, and the intermembrane water layer. This accumulation of effects inhibits access to the detailed dynamical information of each region, and the use of the traditional MSD procedure should thus be avoided. Alternatively, in a local strategy, each spatial region is assumed to be locally homogeneous. The MSD is determined for short time intervals, and a local fit is done in every spatial region, or alternatively, the local diffusivity is related to local force fluctuation correlations,³ or the local mean first passage times.⁴ The local strategy has been applied to water and ion transport through membranes.^{3,5–7}

This paper drops the assumption of the (local) homogeneous isotropic medium, and returns to the original Smoluchowski equation describing diffusion on a free energy surface in an anisotropic and inhomogeneous medium.⁸ For a periodic layered medium, the Smoluchowski equations may be simplified considerably, because of the translational symmetry parallel to the surface. The resulting two equations, given below, depend on a normal and radial diffusion profile and can be cast into a series of one-dimensional diffusion equations with sink terms.

For one-dimensional diffusion, Bayesian inference has been used previously to extract free energy and diffusion profiles from molecular dynamics trajectories.^{9,10} To sample from the Bayesian posterior, the profiles are varied randomly with Monte Carlo moves, and the probability to accept a new profile is determined by the likelihood of the profiles to explain the observed trajectory. In this way, one obtains free energy and diffusion profiles that can best account for the observed simulation trajectories. This method works well for the one-dimensional Smoluchowski equation.⁹ For the multidimensional problem of rotational and translational pair diffusion, a representation in terms of basis functions has made the problem numerically tractable.¹¹ We will show in the Methodology section that this procedure is also extendable to a periodic layered medium, such as a membrane, by adapting the equation with a sink term, and by using a series of Bessel functions as basis functions. This is the major theoretical result of the paper.

Using the new methodology, we have investigated O₂ diffusion through two membrane models with molecular simulations, as shown in Fig. 2. One membrane consists of a single lipid type, i.e., 1-palmitoyl 2-oleoyl phosphatidylcholine (POPC). The other membrane models the inner mitochondrial membrane (referred to as MITO) and thereby consists of a variety of lipid types, though no proteins are included for simplicity. Comparison of these two membranes yields insight into the effects of membrane lipid composition on oxygen diffusion. Moreover, the diffusion profiles can lead to the visualization of the oxygen time evolution, as well as to the calculation of the membrane permeability. Such quantitative results are required for models of oxygen trapping by COX.

The paper is organized as follows. The Methodology section starts with the treatment of the Smoluchowski equation to describe diffusion in a periodic layered medium. The discretization of the rate equations gives an expression for the theoretical propagator, which may serve in the likelihood function. The likelihood is sampled in a Monte Carlo procedure, aiming to match the theoretical propagator with the observed propagation in the O₂ trajectories. For the validation, two homogeneous systems were investigated, pure water and pure hexadecane, the latter being a model for the lipid tail region of the membrane. For the validation with an inhomogeneous and anisotropic medium, a hexadecane layer surrounded by water was simulated (top of Fig. 2). In the Results section, the normal and radial free energy and diffusion profiles are compared for the POPC and MITO model membranes, the anisotropy of the bilayer is quantified, and the membrane permeability is derived. Compartmental models are used to compare the permeability with Overton's rule, and to investigate the influence of the small free energy barrier in the head group region and the free energy well in the tail region. The Conclusion section summarizes the main findings of the paper.

II. METHODOLOGY

A. Normal and radial diffusion in a periodic layered medium

Smoluchowski equation in layered medium.—We describe the diffusion of a tracer particle in an anisotropic and inhomogeneous medium by the Smoluchowski equation,

$$\frac{\partial p(\vec{r}, t)}{\partial t} = \nabla \cdot \left[\bar{\bar{D}}(\vec{r}) e^{-\beta F(\vec{r})} \nabla \left(e^{\beta F(\vec{r})} p(\vec{r}, t) \right) \right], \quad (1)$$

where $p(\vec{r}, t)d\vec{r}$ is the spatial probability distribution of the particle as a function of time t . Both the diffusion tensor $\bar{\bar{D}}(\vec{r})$ and the free energy $F(\vec{r})$ depend on the position $\vec{r}$. The free energy may be thought of as the potential of mean force felt by the diffusing particle, with $\beta = 1/k_B T$ the inverse temperature, k_B Boltzmann's constant, and T the absolute temperature. ∇ is the gradient with respect to $\vec{r}$, and $\nabla \cdot$ indicates the divergence.

For a horizontally layered medium, translational symmetry in the xy -plane simplifies the diffusion equation. In particular, both the free energy profile and the diffusion tensor depend solely on the vertical position z , the latter being diagonal in Cartesian coordinates x, y, z ,

$$F(\vec{r}) = F(z) \quad (2)$$

$$\bar{\bar{D}}(\vec{r}) \equiv \bar{\bar{D}}(z) \equiv \begin{pmatrix} D_{\parallel}(z) & 0 & 0 \\ 0 & D_{\parallel}(z) & 0 \\ 0 & 0 & D_{\perp}(z) \end{pmatrix} \quad (3)$$

where $D_{\parallel}(z)$ and $D_{\perp}(z)$ are the z -dependent diffusion coefficients within and normal to the xy layers, respectively. Substituting Eqs. 2 and 3 into Eq. 1, we obtain the Smoluchowski equation for tracer diffusion in a layered medium,

$$\frac{\partial p}{\partial t} = D_{\parallel}(z) \left(\frac{\partial^2 p}{\partial x^2} + \frac{\partial^2 p}{\partial y^2} \right) + \frac{\partial}{\partial z} \left[D_{\perp}(z) e^{-\beta F(z)} \frac{\partial}{\partial z} (e^{\beta F(z)} p) \right]. \quad (4)$$

Theoretical propagator.—To exploit the layer symmetry of the problem, we rewrite the diffusion equation in cylindrical coordinates (r, z, θ) ,

$$\frac{\partial p}{\partial t} = D_{\parallel}(z) \left[\frac{1}{r} \frac{\partial}{\partial r} \left(r \frac{\partial p}{\partial r} \right) + \frac{1}{r^2} \frac{\partial^2 p}{\partial \theta^2} \right] + \frac{\partial}{\partial z} \left[D_{\perp}(z) e^{-\beta F(z)} \frac{\partial}{\partial z} (e^{\beta F(z)} p) \right] \quad (5)$$

where $p = p(\vec{r}, t) = p(r, z, \theta, t)$. The solution of Eq. 5 is the Green's function if the initial condition is a delta peak of concentration at a vertical position

z_0 , $p(\vec{r}, t=0) = \delta(x)\delta(y)\delta(z-z_0) = \delta(r)\delta(z-z_0)/2\pi r$, where $\delta(\dots)$ is Dirac's delta function.

Because of translation symmetry, the choice of origin in the xy plane is arbitrary. Moreover, for the delta initial condition the solution is independent of the polar angle θ , i.e., $p(\vec{r}, t) = p(r, z, t)$. This further simplifies the Smoluchowski equation, turning it into a separable differential equation,

$$\frac{1}{D_{\parallel}(z)} \frac{\partial p}{\partial t} = \widehat{\mathcal{A}}_r p + \widehat{\mathcal{A}}_z p \quad (6)$$

with the operators $\widehat{\mathcal{A}}_r$ and $\widehat{\mathcal{A}}_z$ acting on the r and z coordinates, respectively,

$$\widehat{\mathcal{A}}_r = \frac{1}{r} \frac{\partial}{\partial r} \left(r \frac{\partial}{\partial r} \right), \quad (7)$$

$$\widehat{\mathcal{A}}_z = \frac{1}{D_{\parallel}(z)} \frac{\partial}{\partial z} \left[D_{\perp}(z) e^{-\beta F(z)} \frac{\partial}{\partial z} (e^{\beta F(z)} p) \right]. \quad (8)$$

The form of Eq. 6 suggests a separation of variables. Making the ansatz $p(r, z, t) = \mathcal{R}(r)Z(z, t)$ and dividing by $\mathcal{R}Z$, we obtain

$$\frac{1}{Z} \frac{1}{D_{\parallel}(z)} \frac{\partial Z}{\partial t} - \frac{1}{Z} \widehat{\mathcal{A}}_z Z = \frac{1}{\mathcal{R}} \widehat{\mathcal{A}}_r \mathcal{R} = -\alpha^2, \quad (9)$$

which leads to two differential equations for each value of α^2 ,

$$\frac{1}{r} \frac{\partial}{\partial r} \left(r \frac{\partial \mathcal{R}_\alpha}{\partial r} \right) + \alpha^2 \mathcal{R}_\alpha = 0 \quad (10)$$

$$\frac{\partial Z_\alpha(z, t)}{\partial t} = \frac{\partial}{\partial z} \left[D_\perp(z) e^{-\beta F(z)} \frac{\partial}{\partial z} \left(e^{\beta F(z)} Z_\alpha(z, t) \right) \right] - \alpha^2 D_\parallel(z) Z_\alpha(z, t). \quad (11)$$

Eq. 10 describes the textbook example of radial diffusion (in separated variables) with $\alpha^2 > 0$ (so $\alpha \in \mathbb{R}$), whose relevant solution is the zero-th order Bessel function of the first kind (integration constant omitted),

$$\mathcal{R}_\alpha(r) \sim J_0(\alpha r). \quad (12)$$

Eq. 11 is a one-dimensional diffusion equation with an added sink term $-\alpha^2 D_\parallel(z) Z_\alpha(z, t)$. We can think of this equation as describing the diffusion from layer to layer, but some concentration is lost into low- α modes because of concentration spreading out in the radial direction with the local radial diffusion coefficient $D_\parallel(z)$. To obtain the general solution of Eq. 6, we integrate over all possible values of α ,

$$p(r, z, t) = \int_0^\infty d\alpha J_0(\alpha r) Z_\alpha(z, t). \quad (13)$$

The initial condition on $p(r, z, t)$ determines $Z_\alpha(z, 0)$. We start from the Green's function initial condition $p(r, z, 0) = \delta(r) \delta(z - z_0) / 2\pi r$,

$$p(r, z, 0) = \int_0^\infty d\alpha J_0(\alpha r) Z_\alpha(z, 0) = \frac{\delta(r)}{2\pi r} \delta(z - z_0). \quad (14)$$

Multiplying by $r J_0(\alpha' r)$ and integrating over r gives

$$\int_0^\infty dr \int_0^\infty d\alpha r J_0(\alpha r) J_0(\alpha' r) Z_\alpha(z, 0) = \int_0^\infty dr \frac{J_0(\alpha' r)}{2\pi} \delta(r) \delta(z - z_0). \quad (15)$$

By exchanging the order of integration, and using the orthogonality of the Bessel function and the properties of Dirac's δ -function, we find

$$\int_0^\infty d\alpha \frac{\delta(\alpha - \alpha')}{\alpha} Z_\alpha(z, 0) = \frac{J_0(0)}{2\pi} \delta(z - z_0) \quad (16)$$

which gives the initial conditions for the Z_α functions

$$Z_\alpha(z, 0) = \frac{\alpha}{2\pi} \delta(z - z_0). \quad (17)$$

In practice, the integral over α in Eq. 13 is approximated by setting an upper boundary on the radius r (where limitations of this approximation are described below). Specifically, we require that at some fixed large distance s the probability dies to zero, $p(s, z, t) = 0$. Eq. 13 then requires that $J_0(\alpha s) = 0$. This in turn reduces the integral over α in Eq. 13 to a summation over those values α_k for which $\alpha_k s$ is a root of the J_0 Bessel function. Writing the k 'th root of $J_0(x)$ as x_k , the propagator can be expressed as an infinite sum over the roots,

$$p(r, z, t|z_0) = \sum_{\alpha_k s = x_k} J_0(\alpha_k r) Z_{\alpha_k}(z, t|z_0). \quad (18)$$

The initial condition becomes

$$p(r, z, 0) = \sum_{\alpha_k s = x_k} J_0(\alpha_k r) Z_{\alpha_k}(z, 0) = \frac{\delta(r)}{2\pi r} \delta(z - z_0). \quad (19)$$

Multiplying by $r J_0(\alpha_k r)$ and integrating from 0 to s ,

$$\sum_{\alpha_k s = x_k} \int_0^s dr r J_0(\alpha_k r) J_0(\alpha_k r) Z_{\alpha_k}(z, 0) = \int_0^s dr \frac{\delta(r)}{2\pi} J_0(\alpha_k r) \delta(z - z_0) \quad (20)$$

and exploiting the discrete orthogonality of the Bessel functions, yields

$$\sum_{\alpha_k s = x_k} \frac{1}{2} s^2 J_1^2(x_k) \delta_{kk'} Z_{\alpha_k}(z, 0) = \frac{1}{2\pi} J_0(0) \delta(z - z_0) \quad (21)$$

where the Kronecker delta reduces the sum to the term $k = k'$. This gives the initial condition for Z_{α_k} ,

$$Z_{\alpha_k}(z, 0) = \frac{1}{\pi s^2 J_1^2(x_k)} \delta(z - z_0). \quad (22)$$

Discretized theoretical propagator.—Solving the equations for Z_α (Eq. 11) satisfying the initial condition (Eq. 17) yields the theoretical propagator in Eq. 18. In practice, the space-discretized version of the propagator is used. In the one-dimensional case,^{9,10} the probability p_i of being in a bin i satisfies the differential equation

$$\frac{dp_i(t)}{dt} = \sum_j R_{ij} p_j(t) \quad (23)$$

with R the tridiagonal rate matrix depending on the free energy and diffusion profiles (whose right and lower left corners might be adapted to impose periodic boundary conditions).⁹ The solution in case of a delta peak in bin j at time zero ($p_i = \delta_{ij}$) may be readily written in terms of a matrix exponential

$$p_i(t) = [e^{Rt}]_{ij}. \quad (24)$$

Eq. 11 for the Z_α corresponds to one-dimensional diffusion with a probability sink proportional to Z_α . In the corresponding space-discretized rate matrix, this sink alters only the diagonal elements^{11,12},

$$R_{ii} \mapsto R_{ii} - \alpha^2 D_{\parallel,i}. \quad (25)$$

Using a discretized initial condition ($\delta(z - z_0) \mapsto \delta_{ij}$), the discretized solution for the Z_{α_k} is

$$Z_{\alpha_k}(i, t | j) = \frac{1}{\pi s^2 J_1^2(x_k)} \left[e^{Rt - \alpha_k^2 \mathcal{D}t} \right]_{ij} \quad (26)$$

and the discretized propagator is then

$$p(r, i, t | j) = \sum_{\alpha_k} \frac{J_0(\alpha_k r)}{\pi s^2 J_1^2(x_k)} \left[e^{Rt - \alpha_k^2 \mathcal{D}t} \right]_{ij} \quad (27)$$

where the diagonal matrix $\mathcal{D}$ has the $D_{\parallel,i}$ as diagonal entries, and R uses the discretization scheme of Bicout and Szabo.¹²

It is convenient to work with the probability density $\tilde{p}(r, z, t) = 2\pi r p(r, z, t)$, where the θ dependence is integrated out and the Jacobian of the coordinate transform is taken into account ($dV = dx dy dz = r dr dz d\theta$). The probability to be in a ring-shaped volume element $dV = 2\pi r dr dz$ at vertical position z and axial distance r is then given by $\tilde{p}(r, z) dr dz$.

Finally, the radial coordinate r is discretized. The probability for the particle to be in r -bin m of width Δr around radial position r_m and in z -bin i is given by

$$\tilde{p}(m, i, t | j) = \frac{2r_m \Delta r}{s^2} \sum_{\alpha_k} \frac{J_0(\alpha_k r_m)}{J_1^2(x_k)} [e^{Rt - \alpha_k^2 \mathcal{D}t}]_{ij}. \quad (28)$$

The preceding probability is dimensionless, and it depends on the diffusion and free energy profiles through the matrix elements of R and $\mathcal{D}$. In the maximum likelihood or Bayesian approach, the profile values $R(z)$, $D_{\perp}(z)$, and $D_{\parallel}(z)$ are iteratively varied using a Monte Carlo procedure until an optimal correspondence is obtained between this theoretical propagator and the transitions between bins observed in the trajectories.

In practice, the sum in Eq. 18 is limited to the first $k_{\max}$ Bessel zeros, which introduces a truncation error. Each Bessel function $J_0(\alpha_k r)$ starts at 1 for $r = 0$ and oscillates with k zeros in the $[0, s]$ interval. The truncated sum of Bessel functions therefore might be negative in some r -intervals, whereas the probability distribution should always be positive. This artifact of unwanted negative values leads to instabilities in the log-likelihood evaluation. It is advisable to set a threshold on the lower end in the Monte Carlo procedure, such that the probability defaults to some small cutoff value (e.g., 10^{-7}) when the truncated Bessel sum falls below the cutoff. In addition, the imposition of $\mathcal{R}(s) = 0$ corresponds to an absorbing outer radial boundary. Therefore, at times $t > 0$ the probability in Eq. 28 summed over m and i should be normalized to one to compensate for any flux out of the domain $r < s$.

Propagator for normal diffusion.—Because of the layer symmetry, one can construct an equation for diffusion along z that is independent of r . We define a propagator Q for the normal diffusion by integrating the full propagator over the xy plane. In terms of the polar coordinates r and θ , we have

$$Q(z, t) = \int_0^\infty dr \int_0^{2\pi} d\theta r p(r, z, t) = \int_0^\infty dr \tilde{p}(r, z, t). \quad (29)$$

Multiplying Eq. 5 by $r dr d\theta$, and again integrating θ from 0 to 2π and r from 0 to ∞ , we obtain a diffusion equation for Q alone,

$$\frac{\partial Q(z, t)}{\partial t} = \frac{\partial}{\partial z} \left[D_{\perp}(z) e^{-\beta F(z)} \frac{\partial}{\partial z} (e^{\beta F(z)} Q(z, t)) \right] \quad (30)$$

where the r -dependent terms proportional to $D_{\parallel}(z)$ cancel because the boundary terms vanish in the partial integrations. Eq. 30 for Q is a position-dependent one-dimensional diffusion equation. With initial condition $Q(z, 0) = \delta(z - z_0)$, $Q(z, t|z_0)$ becomes the propagator in the vertical direction. The corresponding discretized propagator is obtained following the procedure for one-dimensional diffusion,¹²

$$Q(i, t|j) = \left[e^{Rt} \right]_{ij} \quad (31)$$

with R the same rate matrix as above (Eq. 24).

The theoretical propagators Q and $\tilde{p}$ may be used to extract free energy and diffusion profiles from MD trajectories. In the Bayesian analysis, the profiles are constructed by sampling the likelihood function (see Sec. II D). This propagator Q only depends on $R(z)$ and $D_{\perp}(z)$, not on $D_{\parallel}(z)$. Therefore, one can in a first step focus on this one-dimensional propagator Q and extract information on F and $D_{\perp}$, and in a second step proceed to the multidimensional propagator $\tilde{p}$ to extract information on $D_{\parallel}$.

B. Model systems

Five systems are considered (Table I). The Bayesian procedure described in Section IIA is first tested on three simple systems: (1) oxygen diffusion in pure water (WAT), (2) oxygen diffusion in pure n-hexadecane (HEXD), representing the hydrophobic tail region of the membrane, and (3) oxygen diffusion in a slab of n-hexadecane embedded in water (HEXD/WAT), to model the hydrophobic/hydrophilic boundary. Oxygen diffusion in the pure phases can be computed with regular MSD as these systems are homogeneous and offer a first validation of the Bayesian implementation.

For simplicity and to optimize sampling, ten oxygen molecules are included in each system. The experimental solubilities of oxygen in hexadecane and in water are $10.64 \cdot 10^{-3}$ and $1.298 \cdot 10^{-3}$ mol/l/atm, respectively,¹³ implying 1 oxygen molecule per 320 hexadecane molecules or 1 per 42000 water molecules, i.e., substantially smaller than the number included here. Nevertheless, no clustering of oxygen or other artifacts were observed in the simulations, which suggests that no significant dependence on oxygen concentration is expected for the diffusivities.

Two membrane systems are then considered: (4) a bilayer containing the common phospholipid POPC, and (5) a bilayer representing the lipid composition of the mitochondrial inner membrane, including cardiolipin (MITO), which is thought to play a key role in mitochondrial processes.¹⁴ Each membrane contains 36 lipids per leaflet and is surrounded by a $30 \text{ \AA}$ water layer, and periodic boundary conditions are applied.

The composition of mitochondrial membranes depends on the cell type (e.g., heart, lung, plants).¹⁵ The most common lipid head groups of the inner mitochondrial membrane in yeast are phosphatidylcholine (PC, 38.4%), phosphatidylethanolamine (PE, 24.0%), phosphatidylinositol (PI, 16.2%), phosphatidylserine (PS, 3.8%), and cardiolipin (CL, 16.1%).¹⁶

An earlier paper reports the detailed distribution of the phospholipids in pig heart mitochondrial membrane, which could be representative for human heart mitochondrial membrane.¹⁷ Given the low occurrence of PI and absence of PS in this membrane¹⁷ we chose to include only three head types in our MITO model system: PE, PC, and CL. These heads are combined with fatty acid tails according to the reported tail distribution per lipid head.¹⁷ C16:0 and C18:0 are saturated fatty acids with 16 and 18 carbon atoms, respectively. C18:2 has 18 carbon atoms and two double bonds, while the C20:4 arachidonoyl chain has 20 carbons and four double bonds. This leads to four lipids in the MITO system: 1-stearoyl-2-arachidonoyl-sn-glycero-3-PE (SAPE), 1-stearoyl-2-linoleoyl-sn-glycero-3-PE (SLPE), 1-palmitoyl-2-linoleoyl-sn-glycero-3-PC (PLPC), and tetralinoleoyl-CL (TLCL).

The CHARMM36 lipid force field¹⁸ and modified TIP3P water model^{19,20} were used in all simulations. The oxygen model consists of two uncharged particles, whose force field parameters are given in the Supp. Info.

C. Molecular dynamics

The WAT, HEXD, and HEXD/WAT systems were equilibrated using NPAT molecular dynamics simulations in the CHARMM simulation package,²¹ with constant number of atoms N , surface area A in the xy plane, constant normal pressure P , and constant temperature. The pressure was set to 1 atm and the temperature to 310 K, i.e., body temperature. The a and b lattice vectors were fixed to 50 Å, and the unit cell was kept tetragonal. The c lattice vector fluctuated and was on average about 50 Å as well. The leap-frog integrator was used with an integration step of 1 fs to solve the equations of motion of the extended system. The mass of the pressure piston in the z -direction was 800 amu, and the mass of the thermal piston (Hoover thermostat) was 8000 kcal/mol/ps².

The HEXD/WAT system was equilibrated for 15 ns in the NPAT ensemble. The average value $\langle c \rangle$ (taken over the last 14 ns) was used in the subsequent NVT simulation, where the unit cell was completely fixed. The NVT simulation has the advantage that the bin size Δz is constant throughout the simulation, which simplifies the post-processing (Sec. IID). An extension to NPT simulations is in principle possible, which will be tested and implemented for future work. The starting point of the NVT simulation was a snapshot with a volume that lies very close to the average volume and that was scaled to the correct value. The production run lasted 50 ns for the WAT and HEXD systems, during which the coordinates were sampled every picosecond. Four independent trajectories of 50 ns each were created for the HEXD/WAT system, totalling 200 ns of data.

The POPC and MITO systems were equilibrated for 50 ns using NPT simulations, such that the area per lipid could fluctuate as well. The piston mass was 1000 amu in each direction. The temperature was set to 310 K (body temperature) and the pressure to 1 atm. The crystal type was set to tetragonal such that the a and b lattice vectors have equal length. The average lattice vector lengths were then kept constant in the subsequent NVT production run. Four uncorrelated production runs of 50 ns each were created by starting from different snapshots of the NPT run, such that the total simulation time per system is 200 ns. The $z = 0$ plane is chosen at the middle of the hexadecane slab or of the bilayer.

D. Post-processing of trajectories: Bayesian analysis

In the Bayesian analysis, the underlying physical model M of the molecular system is estimated from the observed trajectories T by sampling the likelihood function. The likelihood $L(M)$ of a model M is the probability $P(T|M)$ to observe the trajectories T given that specific model: $L(M) = P(T|M)$. The physical ‘model’ consists here of the free energy and diffusion profiles. In Bayesian analysis, the likelihood is sampled by performing a random Monte Carlo walk in parameter space, where the model parameters are the free energy profile and diffusion profiles. The Monte Carlo search in parameter space, sampling F and $\ln D$ uniformly, consistent with an uninformative scale-invariant prior, generates a distribution of models. The average of these models can be used as an estimate of the optimal model, and the distribution width of the models quantifies the accuracy of the estimate.

In the data analysis of the molecular dynamics trajectories, $n = 100$ bins are created in the z -direction, normal to the surface, with width Δz between 0.54 and 0.7 Å. The discretization of the profiles gives as model parameters the free energy F_i in bin i , the normal diffusivity

$D_{\perp, i+\frac{1}{2}}$ between bin i and $i+\frac{1}{2}$, and the radial diffusivity $D_{\parallel, i}$ in the plane of bin i . The

logarithm of the D values are varied such that all D values are automatically kept strictly positive. Instead of using these individual $3n$ discretized profile values as model parameters, basis functions are introduced to smooth the profiles and to exploit the expected symmetry with respect to $z = 0$. The F and D profiles are periodic and symmetric about $z = 0$, and are thus expressed as a Fourier cosine series. The free energy becomes

$$F_i = a_0 + \sum_{k=1}^{n_b} a_k \cos(2\pi k i / n) \quad (32)$$

and similar expressions can be written for $\ln D_{\perp, i+\frac{1}{2}}$ and $\ln D_{\parallel, i}$. The a_0 parameter may

be set to zero for the free energy, as $F(z)$ is determined up to a constant. The coefficients such as a_k are now the quantities that are varied in the Monte Carlo procedure. The procedure with basis functions allows for a higher number of bins which reduces the discretization error, while avoiding overfitting given the limited number of parameters. In our analysis, 9 coefficients are used for the F profile ($a_0 = 0$ and $n_b = 9$) and 6 coefficients for the $D_{\perp, i+\frac{1}{2}}$ and $\ln D_{\parallel, i}$ profiles each ($a_0 = 0$ and $n_b = 5$). A selection of profiles with a

different number of basis functions is included in the Supp. Info. to show that the chosen numbers n_b are sufficiently high to include the features of the profile without overfitting.

For the normal diffusion, the propagator $Q(i, t | j)$ in Eq. 31 expresses the probability of the particle being in bin i after a lag time t knowing that it was in bin j at the start. From Q (depending on F and $D_{\perp}$), the rate matrix R is constructed, and the likelihood reads

$$\ln L(M) = \sum_{i \leftarrow j} \ln \left(\left[(e^{Rt})_{ij} \right]^{N_{ij}(t)} \right) \quad (33)$$

with the sum running over all possible transitions $i \leftarrow j$ between bins and $N_{ij}(t)$ the observed number of such transitions at a lag time t in the simulated oxygen trajectories. The free energy and normal diffusion profile are constructed by Bayesian inference on the basis of this likelihood. For the model parameters F_i and $D_{\perp, i + \frac{1}{2}}$, which enter through the rate matrix R , we use uniform priors for F_i and for $\ln D_i$. The initial guess for the profiles is a flat free energy profile and a flat diffusion profile of $1 \text{ \AA}^2/\text{ps}$. The coefficients are equilibrated in an initial Monte Carlo run of 50000 steps. In the subsequent production run of 100000 Monte Carlo steps, data are collected every 100 steps, giving 1000 models in total. The reported profiles are the average models with the standard deviations as error bars.

For radial diffusion, the propagator $\tilde{p}(m, i, t | j)$ in Eq. 28 expresses the probability of the particle being in z -bin i and radial bin m after a lag time t , given that it was in z -bin j and $r=0$ at the start. Similarly to normal diffusion, the logarithm of the likelihood is calculated with this propagator,

$$\ln L(M) = \sum_{m, i \leftarrow j} \ln \left(\left[\Delta r \sum_{\alpha_k} 2r_m \frac{J_0(\alpha_k r_m)}{s^2 J_1^2(x_k)} \left[e^{Rt - \alpha_k^2 D t} \right]_{ij} \right]^{N_{mi, j}(t)} \right) \quad (34)$$

with the first sum running over all possible transitions $m, i \leftarrow j$ between bins and $N_{mi, j}(t)$ the observed number of such transitions at a lag time t in the trajectories. This likelihood function is sampled with Bayesian analysis in order to determine the radial diffusion profile. While the coefficients for $D_{\parallel, i}$ are varied, the free energy and normal diffusion profiles are kept constant during both the (slow) equilibration run of 100000 Monte Carlo steps and the production run of 100000 steps. The production run takes approximately 2 hours and 100 hours on a single CPU for each normal and radial profile, respectively. The distance s at which the propagator becomes zero is set to $50 \text{ \AA}$, the radial distance r is discretized into 100 bins of size $r = 0.5 \text{ \AA}$, and the number of Bessel functions is set to $k_{\max} = 50$ functions.

III. RESULTS AND DISCUSSION

A. Oxygen diffusion in water, hexadecane, and hexadecane/water

Selecting a time scale.—To construct the likelihood according to Eq. 33, the transitions between bins over a lag time t should be counted. The choice of an appropriate lag time is here based on an analysis of oxygen diffusion in the neat systems, WAT and HEXD. The lag time should be sufficiently long to be sensitive to the oxygen diffusion process, for oxygen molecules to explore beyond their local cages. However, long lag times make the matrix exponential in Eq. 33 insensitive to diffusion information. In the limit of large t , the

probability to find an O₂ molecule at a certain location is the equilibrium distribution determined by the free energy profile, independent of differences in diffusivity to reach this equilibrium distribution. Both for WAT and HEXD, we obtain an accurate fit of a simple diffusion model in which the MSD grows as $b + 6Dt$ for $t > 10$ ps, with b an offset that accounts for an initial fast spread for $t < 10$ ps (Fig. 3). The offset b is quite small for WAT, and more significant for HEXD. By adding the line $6Dt$ in Fig. 3, the turnover in the double logarithmic plot seems to lie higher, at about 100 ps for HEXD. While this might be interpreted as “sub-diffusive” behavior at short times, this is deceiving and largely explained by the offset $b > 0$.

Fitting the slope of the MSD(t) curve in the equation $MSD = b + 6Dt$ in a range of lag times longer than 10 ps, yields $D = 0.6 \text{ \AA}^2/\text{ps}$ in water and $D = 0.44 \text{ \AA}^2/\text{ps}$ in hexadecane. The Bayesian procedure with imposed flat profiles ($n_b = 0$ in Eq. 32) and lag time 10 ps gives an oxygen diffusivity of $0.633 \pm 0.02 \text{ \AA}^2/\text{ps}$ in neat water and $0.564 \pm 0.02 \text{ \AA}^2/\text{ps}$ in neat hexadecane. The values clearly differ between the Bayesian analysis and the MSD slope for hexadecane. The source of this discrepancy is that the Bayesian model does not discriminate between the short and long time scale and assumes “pure” diffusive behavior. The underlying Bayesian model assumes the proportional relation $MSD = 6Dt$, while an offset b is allowed when fitting the slope with $MSD = b + 6Dt$.

To remedy the discrepancy, the Bayesian propagators are constructed at multiple lag times t_i giving multiple D_i estimates. By equating $6D_i t_i$ from Bayesian analysis to $b + 6Dt$ (allowing an offset), we find the relation $D_i = D(1 + t_0/t_i)$. Diffusivities D capturing the long-time dynamics are obtained from a linear fit of the Bayesian estimates D_i against $1/t_i$, with fit parameters D and Dt_0 , where t_0 is a time offset. In the WAT, HEXD and HEXD/WAT systems, the selected lag times t_i were 10, 20, 30, and 40 ps for the fit of D . In the POPC and MITO systems the diffusive regime was reached somewhat more slowly (Fig. 3b), and the selected lag times were 20, 30, 40, and 50 ps. (The data and the fits are included in the Supp. Info.) Alternatively, the propagators at multiple lag times could also be analyzed simultaneously in an extended model including a shift in the time origin to account for deviations from diffusive behavior at short times.¹¹ This was not pursued here to limit the number of free parameters. The Bayesian diffusion coefficient obtained by using this fitting procedure is $D = 0.603 \text{ \AA}^2/\text{ps}$ in water and $D = 0.455 \text{ \AA}^2/\text{ps}$ in hexadecane, giving a close agreement with the fit to the slope of the MSD. This shows the equivalence of the standard MSD method and the Bayesian approach for a homogeneous medium.

The free energy profiles were insensitive to the chosen lag time in the Bayesian analysis. The reported F profiles (see further) were constructed with a lag time of 10 ps for HEXD/WAT and 20 ps for the POPC and MITO systems.

Comparison of simulated and experimental diffusivities in neat systems.—All of the preceding diffusion coefficients from simulations are larger than in experiment,¹³ as would be expected from the deficiencies in the water and alkane force fields. The Supp. Info. contains a set of relevant experimental and simulated values.^{13,23–26} In summary, the difference between experimental and simulated values is due to differences in viscosity (the force field), temperature, and experimental errors.

First, consider the oxygen diffusion coefficient in neat water. The experimental value is $0.211 \text{ \AA}^2/\text{ps}$ at 22°C , as measured by Ju et al.¹³ The simulations at 310 K overestimate the oxygen diffusion by a factor 2.9. This is mostly due to a difference in viscosity between experiment and simulations, i.e. the water force field. The experimental water viscosity is 1.002 cP at 20°C ,^{23,24} while the simulated water viscosity is 0.329 cP at 20°C ,²⁵ also showing a difference of a factor 3. The difference is also due to temperature, where the experimental viscosity is somewhat more sensitive to temperature than the simulated viscosity.

Second, consider the oxygen diffusion coefficient in neat hexadecane. The experimental value is $0.249 \text{ \AA}^2/\text{ps}$ at 22°C .¹³ The simulations at 310 K overestimate the oxygen diffusion by a factor 1.8. The difference in diffusivities is again mostly due to a difference in viscosity between experiment and simulations, i.e. the hexadecane force field. In our simulations of hexadecane, we found a viscosity of 1.6 cP at 310 K. At 22°C , the experimental hexadecane viscosity is 3.33 cP ,²⁶ and the simulated viscosity is 2.2 cP , showing a difference of a factor 1.5. The sensitivity to temperature is similar between experiment and simulations for hexadecane, such that the remaining difference in oxygen diffusivity should be due to the force field and experimental errors (a factor two is common). Moreover, the diffusion mechanism in alkanes is a hopping mechanism, such that diffusivities not necessarily track viscosities.

Comparison of free energy profiles.—The HEXD/WAT system is a simple model system for oxygen diffusion in a lipid bilayer. The hexadecane layer is a hydrophobic region representing the lipid hydrocarbon tails and is surrounded by a water environment. The free energy profile (blue line) in Fig. 4 was obtained from Bayesian analysis. The center layer ($|z| < 8 \text{ \AA}$) should resemble bulk hexadecane, while the outer layer ($|z| > 20 \text{ \AA}$) should resemble bulk water. According to Fig. 4, the free energy difference $F_{\text{w/h}}$ for O_2 residing in bulk water versus bulk hexadecane is $2.9 k_B T$, which means that the oxygen solubility is about 18 times higher in hexadecane than in water with the current force field. This estimate assumes that the interface has no effect on the hexadecane and water phases in the HEXD/WAT model system. (The experimental value of the partition coefficient $[\text{O}_2 \text{ in hexadecane}]/[\text{O}_2 \text{ in water}]$ at 22°C equals 8.2,¹³ approximately half that of the value obtained here at 37°C .) The interface between hexadecane and water shows a minimum and appears to be more favorable for O_2 than bulk hexadecane by $F_{\text{interf}} = 0.4 k_B T$. This may be explained by the hydrophobic nature of hexadecane, which leads to a lower particle density at the interface (see figure electron density in Supp. Info.), and hence there is simply more space available for the oxygen molecule. As our simulations were long enough to have fully equilibrated, the free energy profiles obtained from Bayesian analysis may be compared to the logarithm of a histogram count $h(z)$ of oxygen z -positions, i.e., $F(z) = -k_B T \ln h(z)$. The two plots in Fig. 4 nearly coincide, confirming the validity of the Bayesian approach to derive $F(z)$. In the following, all reported free energy profiles $F(z)$ were created with Bayesian analysis.

Comparison of diffusion profiles.—Both the free energy profile and normal diffusion coefficient are sampled in the Bayesian analysis of the likelihood function in Eq. 33, where R depends on both F and $D_\perp$. In the subsequent step, the radial diffusion coefficient is extracted using Eq. 34. As shown in Fig. 5, normal diffusion resembles radial diffusion in

the center of the hexadecane layer ($|z| < 5 \text{ \AA}$), and they compare reasonably well with diffusion in pure hexadecane. By contrast, diffusion at the interfaces has a high anisotropy ratio up to $D_{\parallel}/D_{\perp} = 1.6$. This is a consequence of the anisotropy in the local particle packing density, with a relatively low density in the interfaces favoring parallel diffusion. Diffusion in the water layer ($|z| > 15 \text{ \AA}$) is fairly different from the reference value in pure water. The water molecules are more ordered in the first layers of the hydrophobic interface than in bulk water, causing a dip in the normal diffusion. An extended effect of hydrophobicity on the water structure could be responsible for the large variations in the diffusion coefficient and the anisotropy in the quite narrow water layer.

B. Oxygen diffusion in POPC and MITO membranes

Free energy profiles.—Fig. 6a plots the free energy profiles across the POPC and MITO bilayers. The bilayer is a more favorable location for O_2 than the water region with a free energy difference of approximately $3k_B T$, similar to the free energy difference $F_{w/h}$ between the hexadecane and water layers in the HEXD/WAT system. The most favorable location is the interleaflet plane, where the packing density is lowest (see figure electron density in Supp. Info.). To enter the membrane and cross the head group region at $\pm 20 \text{ \AA}$, oxygen encounters a barrier of $1 k_B T$ in POPC and a slightly lower barrier of $0.6 k_B T$ in MITO. Crossing the head group region for oxygen is therefore somewhat easier in the MITO membrane than in the POPC membrane.

The shape of the free energy profile looks very similar to the profile through dipalmitoylphosphatidylcholine (DPPC) lipid membrane and the branched DPPC membrane simulated with another force field at 323 K.⁵ They also show the features of the slight preference of oxygen for the interleaflet plane over the tail region, and the small free energy barrier at the interfacial region.⁵ Cordeiro²⁷ reports a similarly shaped profile for POPC at 310 K and a barely noticeable free energy barrier in the head group region, albeit on the basis of another force field and umbrella sampling in the NPT ensemble, with adapted oxygen van der Waals parameters to reproduce its solvation free energy in both water and hexadecane. The free energy barrier is about 0.5 to $1 k_B T$ in the DPPC simulations by Holland et al.²⁸ using the same lipid force field as in the present paper, but at different temperatures of 323 and 350 K, while Marrink and Berendsen²⁹ report no free energy barrier at 350 K using a different force field for DPPC.

Diffusion and anisotropy.—Figs. 6c-d plot the normal and radial diffusion profiles. The shapes of both normal and radial diffusion profiles are similar, with maxima in the water layer and at the center of the bilayer. In the head group and tail regions, we observe a broad dip in the profiles. The average of approximately $0.2 \text{ \AA}^2/\text{ps}$ is close to the recently obtained experimental values of Moller et al. for fluid phase bilayers.³⁰ The range of diffusivities is similar to the work by Al Abdul Wahid et al.,³¹ who simulated a normal diffusion profile in the range 0.2 to $1 \text{ \AA}^2/\text{ps}$ at 318 K for a different type of lipid membrane (MLMPC) using a short lag time and local mean squared displacements. Their profile's shape is different with lower diffusivities in the water layer than in the membrane. This is most likely due to a combination of the temperature difference, lipid composition, methodology of determining the position-dependent diffusion coefficient and, importantly, due to the use of Langevin

dynamics to maintain the temperature. Awoonor-Williams et al. simulated a normal diffusion profile in the same range of diffusivities for DPPC where the diffusivity in water is not as high as in the bilayer.³² Wang et al.³³ constructed a normal and radial diffusion profile for POPE using only 15 ns of data, and likewise, the Langevin thermostat alters the diffusivities.

In our simulations, both normal and radial diffusion barely reach the diffusion levels of oxygen in the neat hexadecane and water layers. It could be that the additional structure in bilayers compared to neat HEXD and WAT liquids reduces the diffusion. The MITO profiles mostly lie above the POPC profiles, meaning that diffusion is faster in the mitochondrial membrane. These differences between POPC and MITO are fairly small compared to the usual accuracy of diffusion-coefficient measurements.

The anisotropy is quantified by the ratio of $D_{\parallel}$ and $D_{\perp}$ in Fig. 6b. Radial diffusion is faster at the bilayer-water interfaces at $|z| \approx 25\text{--}30 \text{ \AA}$ and at the center of the bilayer, with the anisotropy ratio exceeding one. As a likely explanation, normal diffusion out of the interfaces and the bilayer center is hindered by tight water and lipid head group interactions, and by the packed lipid tails, respectively. By contrast, parallel diffusion is comparably fast because of defects in the interactions between headgroups and water, and across leaflets. In particular, oxygen at the bilayer center may diffuse more easily parallel than orthogonal to the plane because of the reduced packing density in the interleaflet region. In the lipid tail region around 5 to 20 $\text{\AA}$, the anisotropy ratio drops below 1, since oxygen diffuses faster parallel to the alkane chains than orthogonal to the chains.

C. Oxygen permeability of the membrane

Whereas biological membranes form a barrier for ions and water, they are quite permeable to oxygen.³⁴ Only few authors suggest the need for oxygen channels to ensure high-volume transmembrane oxygen flow.³⁵ The transport efficiency through a membrane in the normal direction is expressed by the permeability, which can be derived both from simulations and experimentally. The transport efficiency in the radial direction will be considered in Section IIID).

1. Permeabilities from simulations—The oxygen permeability P through a membrane of thickness h can be derived from the free energy and normal diffusion profile using the equation

$$\frac{1}{P} = e^{-\beta F_{\text{ref}}} \int_{-h/2}^{h/2} \frac{1}{e^{-\beta F(z)} D_{\perp}(z)} dz \quad (35)$$

where F_{ref} is the value of $F(z)$ in the bulk water, far from the membrane.³ $D_{\parallel}$ is not present in the expression, because permeability through the membrane only measures diffusion normal to the membrane. The formula is referred to as the inhomogeneous solubility-diffusion model,³ and the model's assumptions are equivalent to assuming that mass transport is dictated by the Smoluchowski equation for $Q(z, t)$ in Eq. 30. Eq. 35 indeed follows by

determining the stationary flux ($\partial Q/\partial t = 0$) of Eq. 30 under the boundary conditions of a fixed concentration difference q_0 (by imposing $Q(-h/2, t) = q_0$ and $Q(h/2, t) = 0, \forall t$).

Table III lists the computed permeabilities of the membranes based on the profiles of Figs. 4–6. The oxygen permeability for the lipid composition in MITO (Table II) leads to an approximately 40% larger permeability than in the POPC membrane. This does not imply that the mitochondrial membrane is more permeable to O_2 than pure POPC; the mitochondrial membrane proteins and curvature must be included for a definite comparison.

It is instructive to compare the permeability of a membrane with that of a slab of bulk liquid, because it puts the membrane transport properties in perspective and experimental values are available. Bulk liquid has a flat free energy profile $F(z) = F_{\text{ref}}$ and a constant diffusivity D , which simplifies Eq. 35 for a slab of thickness h to

$$P = \frac{D}{h}. \quad (36)$$

A hexadecane slab of equivalent thickness yields a permeability that is about three times that of the membrane. The hexadecane permeability is 15% lower than the water permeability because of its lower diffusivity.

Subczynski et al.^{36,37} obtained permeabilities at 35°C from the so-called experimental oxygen transport parameter profile $W \sim D(z)e^{-\beta F(z)}$, whose inverse W^{-1} is proportional to the permeation resistance per unit length. Their experimental values are $P = 157.4$ cm/s for POPC and $P_{\text{WAT}} = 53.3$ cm/s for a water layer with the same thickness (54 Å) as the membrane.³⁷ Our simulations thus underestimate the preceding POPC permeability and overestimate the water permeability. The experimental permeabilities are sensitive to temperature and bilayer composition, e.g., the presence of cholesterol, and the error is estimated to be approximately 30%.³⁷ Differences among published experimental permeabilities have also been suggested to arise from variations in the experimentally determined diffusivities in the water reference system.^{34,38} For instance, based on the experimental diffusivities by Ju et al.,¹³ the predicted oxygen permeability is $P_{\text{WAT}} = 39.1$ cm/s and $P_{\text{HEXD}} = 46.1$ cm/s for a slab of 54 Å bulk water and bulk hexadecane, respectively, illustrating the uncertainty in permeabilities. Nevertheless, most experimental values lie in the range 10 to 100 cm/s^{28,32,34} (only few authors report lower values, down to 0.004 cm/s³⁵), so the agreement between simulated and experimental permeability values is acceptable for the present test of the method. The deviations in water viscosities between experiment and simulations (cfr. discussion in Sec. III A) also affect the permeabilities. For instance, the simulated P_{WAT} is about two times higher than the experimental value, which is consistent with the simulated water viscosity being a factor of about 2.6 too low at 310 K. Lastly, permeabilities might deviate between simulations and experiment because of differences in the O_2 partition coefficient between membrane and water. In our hexadecane/water mixture simulations, we noted a difference by a factor of two. Clearly, there is room for improvement through better force fields.^{39,40}

As already noted, the permeabilities P of both the POPC and MITO bilayers are less than those of neat water and hexadecane, $P < P_{\text{WAT}}$ and $P < P_{\text{HEXD}}$, which means that both bilayers form a barrier for oxygen in our simulations. The behavior is very different for the HEXD/WAT layer, which is about 9 times more permeable than the lipid membranes (Table III). In contrast to what intuitively might be expected, the HEXD/WAT layer is thus a flawed predictor of bilayer transport properties when used in a molecular dynamics simulation. A homogeneous slab of lipid tails surrounded by water gives too simple a picture of the membrane. The origin of the different behavior in HEXD/WAT is the free energy profile, as will be demonstrated in the following subsection.

2. Compartmental models—Compartmental models can illustrate the role of the free energy and diffusion profiles. In a 1-compartment model, we have a membrane of thickness h with constant diffusivity D and a flat free energy profile $F(z) = F_1$ across the membrane. The permeability in Eq. 35 then incorporates the free energy difference $\Delta F = F_1 - F_{\text{ref}}$ between the membrane and the bulk phase outside the membrane,

$$P = \frac{De^{-\beta\Delta F}}{h}. \quad (37)$$

In this equation, the role of the partition coefficient $K = e^{-\beta\Delta F}$ becomes apparent. When the membrane is a free energy barrier ($F > 0$), the permeability is reduced. When the membrane is a free energy well ($F < 0$), oxygen prefers residing in the membrane over residing in the bulk liquid, and oxygen transport is faster. This explains the different behavior in the systems of Table III. The free energy profile for the HEXD/WAT layer (Fig. 4) has a free energy well with deeper grooves at the interface, leading to higher permeability. The free energy profile for the POPC or MITO bilayers (Fig. 6) has a well in the bilayer center but also a barrier in the head group region, and this yields a net lower permeability. Another case is when hydrophilic molecules diffuse through the membrane. Water molecules, for instance, experience a high free energy barrier, and consequently the water permeability is about 100 times lower than the oxygen permeability. **[GH: such a strong statement should be backed up by references]** Of course, there are no free energy wells and barriers in the neat water or neat hexadecane slabs.

It is now our aim to study the importance of the main features of the free energy and diffusion profile of the bilayers by incorporating the features in a new compartmental model where the parameters may be freely adjusted. The 1-compartment model of Eq. 37 has been used by Overton in the early 20th century and many others.^{41–43} Nagle et al.⁴⁴ presented a 3-compartment model for water transport where the free energy has a trapezoidal shape, with a constant (high) value in the hydrophobic tail region and a continuous slope in the head group region towards the (low) free energy outside the membrane. For oxygen transport, the 3-compartment model contains too few details of the complex free energy and diffusion profiles, and we therefore present here a model with 5 compartments, drawn in Fig. 7.

In our model, three regions are distinguished: the head groups $15 < |z| < 25$ Å (region 1), the lipid tails $2.5 < |z| < 15$ Å (region 2), and the midplane $|z| < 2.5$ Å (region 3). Each region i has approximately a flat diffusion constant D_i and a constant free energy difference F_i between region i and the bulk water phase. From Eq. 35, the inverse of the permeability is

$$\frac{1}{P} = \frac{h_1}{D_1 e^{-\beta \Delta F_1}} + \frac{h_2}{D_2 e^{-\beta \Delta F_2}} + \frac{h_3}{D_3 e^{-\beta \Delta F_3}} \quad (38)$$

where the thicknesses are given by $h_1 = 20$ Å, $h_2 = 25$ Å, and $h_3 = 5$ Å, totalling a layer of 50 Å.

Based on the computed profiles in Fig. 6, the parameters D_i and F_i are estimated, as shown in Table IV. Five models are sketched in Fig. 7. Model **a** is the most realistic one, with three prominent features: (i) a dip in diffusivity in the lipid tail region, (ii) a free energy barrier in the head group region, and (iii) a free energy well in the lipid tail region. The predicted permeability of 25 cm/s compares well to the computed values in Table III for POPC and MITO. The other models investigate the effect of the dip in diffusivity, free energy barrier, and free energy well. The dip in diffusivity has less effect than the free energy in these models (model **b** versus other models). Reducing the free energy barrier in the headgroup region 1 enhances the permeability (model **c** versus model **a**). By contrast, removing the free energy well at the membrane center makes the membrane less attractive and reduces the permeability (models **d/e** versus models **a/b/c**).

D. Radial flux of oxygen in the bilayer

The transport efficiency in the normal direction was measured by the permeability in the previous section, since this quantity is accessible experimentally. The transport efficiency in the radial direction is usually expressed by the average radial diffusion coefficient, $D_{\parallel}^{\text{ave}}$. In experiments, diffusion in the membrane has been measured by fluorescence recovery after photobleaching (FRAP) or by single-molecule tracking of fluorescently labeled groups. While no such experiments are available for oxygen, $D_{\parallel}^{\text{ave}}$ can be directly computed from the free energy and diffusion profiles. The radial flux is the result of diffusion through a set of parallel layers. For such a parallel setup, the average radial diffusion coefficient is the weighted average of the radial diffusion profile $D_{\parallel}(z)$,

$$D_{\parallel}^{\text{ave}} = \frac{\int_{-h/2}^{h/2} e^{-\beta F(z)} D_{\parallel}(z) dz}{\int_{-h/2}^{h/2} e^{-\beta F(z)} dz}. \quad (39)$$

As expected from the weighting in Eq. 39, the $D_{\parallel}^{\text{ave}}$ values in Table III lie between the extrema of the $D_{\parallel}(z)$ profile, and $D_{\parallel}^{\text{ave}}$ is dominated by regions where $F(z)$ is low. According to $D_{\parallel}^{\text{ave}}$, radial oxygen diffusion is slower inside the membrane than in the bulk phases, while

it is higher in the HEXD/WAT system because of the fast oxygen transport in the heavily populated interface region.

We define an effective radial permeability $P_{\parallel}$ to compare radial and normal transport. Consider a patch of membrane with thickness h in the z -direction, length h in the x -direction, and extending infinitely in the y -direction. The radial permeability (parallel to the membrane surface) of this patch is then

$$P_{\parallel} = \frac{D_{\parallel}^{\text{ave}}}{h}. \quad (40)$$

There is no factor $e^{-\beta\Delta F}$, in contrast to Eq. 37, because the free energy is independent of the x -direction (translational invariance parallel to the membrane). While not a permeability in the strict sense, $P_{\parallel}$ nevertheless measures how fast oxygen is transported in the membrane over a distance h parallel to the surface.

The radial flux is fairly similar in the membranes, with $P_{\parallel}$ values of 36 and 39 cm/s in the POPC and MITO membranes, respectively (Table III). The ratio $P_{\parallel}/P$ gives information about the anisotropy between radial and normal fluxes. Radial diffusion is 38% faster than normal diffusion in the POPC membrane and 9% in the MITO membrane. The anisotropy is more explicit and opposite in the HEXD/WAT system, where the radial flux is about half as efficient as the normal flux.

In the compartmental model, the radial permeability of a patch of length h in the x -direction is given by

$$P_{\parallel} = \frac{1}{h} \frac{\sum_{i=1}^3 h_i D_i e^{-\beta\Delta F_i}}{\sum_{j=1}^3 h_j e^{-\beta\Delta F_j}}. \quad (41)$$

where the model is assumed to be locally isotropic with $D_{\perp,i} = D_{\parallel,i} = D_i$ in each compartment. Table IV shows that the radial permeabilities depend less on the details of the free energy profile than the normal permeabilities. This is consistent with the finding that the $P_{\parallel}$ values are fairly similar for the POPC and MITO membranes in Table III. According to Eq. 41, the dominant contributions come from thick layers (h_i large) with low free energy (F_i low) and fast diffusion (D_i high).

E. Propagator for oxygen transport in and through the POPC and MITO membranes

The balance between normal and radial transport is central when considering the flux of oxygen within the membrane, and the efficiency of oxygen delivery to its ultimate sink, membrane-bound cytochrome c oxidase. Fast radial transport increases the effective cross section over which oxygen is captured. Conversely, rapid release of membrane bound oxygen increases the effective resistance of oxygen transport, requiring higher partial

pressures to maintain the overall oxygen flux. Using Fig. 1 as a guide, we first consider the case of slow radial diffusion, $D_{\parallel} \ll D_{\perp}$. In this case, the distance travelled in the membrane plane is small; an oxygen molecule entering the membrane would sample little of the interior before exiting. The concentration of proteins such as cytochrome *c* oxidase would then need to be high to ensure binding of oxygen (or the O_2 concentration is high on both sides of the membrane). Conversely, if $D_{\parallel} \gg D_{\perp}$, an oxygen molecule travels large radial distances before exiting the membrane, and a lower oxygen pressure or oxidase concentration suffice.

The potential of mean force $F(z)$ also modulates oxygen transport. For the membranes simulated here, the pronounced free energy minimum at the bilayer center (Fig. 6a) enhances the population of O_2 in the interleaflet plane. Moreover, the presence of the free energy barrier located at the head group region influences membrane permeabilities strongly. Hence, even though $D_{\parallel}$ and $D_{\perp}$ are comparable at $z = 0$ (Fig. 6c-d), the traveled radial distance is expected to be large with respect to the bilayer thickness because the O_2 is, in effect, trapped in the midplane and has ample time to explore the membrane interior.

The anisotropy of radial and normal fluxes is illustrated by the ratio $P_{\parallel}/P_{\perp}$, as listed in Table III. To provide a more detailed picture of the transport in a layered medium, with oxygen molecules flowing in and out of the membrane while traveling radially, we visualize in Fig. 8 the two-dimensional propagator as a function of time. The initial condition is a probability peak at $z \approx -26$ Å, located just outside the bilayer. The propagator $\tilde{p}(z, r, t | z_0 = -26 \text{ Å})$ of Eq. 28 is plotted for a series of lag times t as a function of the traveled distance r in the radial direction and the z -position.

The initially peaked distribution slowly diffuses into the membrane, slightly slower in the POPC than in the MITO membrane because of the slightly higher free energy barrier ($1 k_B T$ versus $0.6 k_B T$). Part of the oxygen concentration diffuses into the water layer, which was extended with 100 water-like bins to reduce periodic boundary effects. After about 1 ns, oxygen reaches the center of the membrane. By 5 ns the concentration distribution through the membrane has reached its equilibrium, and the peak of the oxygen distribution has meanwhile traveled a radial distance r of about 35 to 65 Å.

IV. CONCLUSION

The separation of variables in normal and radial components in the Smoluchowski diffusion equation leads to a new methodology to analyze the anisotropy of lipid bilayers. The essential step was the derivation of the theoretical propagator $\tilde{p}$ that gives the probability evolution in time based on position-dependent free energy and diffusion profiles. In our methodology, these profiles are optimized in a Monte Carlo routine, with the aim to match the theoretical propagator with the observed oxygen diffusion in atomistic molecular dynamics simulations. This Bayesian analysis gives us the optimal free energy, normal diffusion, and radial diffusion profiles, including their statistical uncertainties. The methodology can be applied to lipid bilayers and any other layered system, and is thus a powerful post-MD analysis tool.

In selecting the lag time t in the Bayesian approach, one faces the problem that diffusive descriptions tend to become accurate only at times long compared to local molecular events, yet at long times the observed dynamics is a convolution of diffusivity and free energy profiles. Practically, one usually varies the lag time to find a regime with results that depend minimally on lag time and are statistically meaningful. We used a reference system, water and hexadecane, to verify that the long time scale behavior sets on after about 10 to 20 ps. To remedy for the effect of the short time scale behavior, the diffusion profile is constructed by fitting profiles obtained with Bayesian analysis at various lag times, thus ensuring that D represents the effective diffusivity governing the translational motion at long times.

As a simplified model of lipid bilayers, a hexadecane slab surrounded by water, was also investigated. The HEXD/WAT system predicts a free energy gain of $2.9 k_B T$ when hydrophobic oxygen resides in hexadecane rather than in water. The normal and radial diffusion profiles resemble diffusion of neat hexadecane in the center of the slab, but show high anisotropy in the interface between the slab and the water. This is a first demonstration that a low particle density at an interface can create anisotropic diffusion. Oxygen preferentially is located at the interface and is more mobile parallel to the slab than normal to the slab because of the easier transport in the free volume at the interface.

The methodology was next applied to a pure POPC membrane and a membrane containing cardiolipin molecules as in the inner mitochondrial membrane. The free energy profiles differ from that of the HEXD/WAT system, with free energy barriers of 0.6 to $1 k_B T$ in the head group region, and the interleaflet region is most favorable. The diffusion profiles also differ significantly from the HEXD/WAT system, with a peak at the center of the membrane and a drastic drop in diffusivity in the lipid tail region. The free volume in the interleaflet region creates substantial anisotropy, as in the interface region of the HEXD/WAT system, and oxygen diffusion is faster parallel than orthogonal to the membrane. Therefore, the HEXD/WAT system does not account for essential transport properties of lipid bilayers.

The POPC and MITO systems have very similar profiles for F , $D_{\perp}$, and $D_{\parallel}$. The free energy barrier is somewhat lower for MITO and the normal and radial diffusion profiles are somewhat higher than for POPC. The membrane permeability P is higher for the MITO model system than for the pure POPC model system. The average radial diffusivity was converted to an effective radial permeability $P_{\parallel}$, and the anisotropy between radial and normal fluxes is reflected by the ratio $P_{\parallel}/P > 1$ for the membranes. In contrast, this ratio lies below 0.5 for HEXD/WAT, confirming that oxygen transport in the HEXD/WAT model system is not representative for transport in membranes. A one-compartment model (consistent with Overton's rule) contains too few details for quantitative predictions of membrane permeability, but a compartmental model with 5 compartments showed that barriers at the interface largely explain the differences between POPC and MITO and the HEXD/WAT systems.

The properties calculated here provide a preliminary model of normal and radial diffusion in cell membranes. While simulations of protein-containing bilayers with more lipid diversity and membrane curvature are required to support and refine the model, the present simulations indicate that oxygen quickly enters the membrane and proceeds to the

interleaflet space in about 1 ns. It then has ample time to diffuse radially, mostly in the interleaflet space, and to interact with membrane proteins containing binding pockets in the center of the membrane, such as cytochrome *c* oxidase.^{1,2}

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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FIG. 1.

Oxygen diffuses into the phospholipid membrane to reach the binding site of cytochrome c oxidase at the center of the bilayer, where it is trapped and consumed (reduced). Trajectories (orange) can exit down (traj. 1), exit up (traj. 2) or lead to reduction on the O_2 binding site (traj. 3). The bilayer is inhomogeneous and anisotropic: the normal ($D_{\perp}$) and radial ($D_{\parallel}$) diffusion coefficients depend on the z -location in the membrane. The center of the bilayer is located at $z=0$, and the membrane thickness is h .

FIG. 2.

Three inhomogeneous and anisotropic systems are investigated: a hexadecane layer surrounded by water (HEXD/WAT, top), a membrane consisting of a single lipid type (POPC, middle), and a model mimicking the lipid composition of inner mitochondrial membrane (MITO, bottom). Each simulation box contains 10 oxygen molecules (red). In the MITO system, some ions are dispersed (light green for Cl^- , purple for K^+) in the water layer, and each leaflet contains 6 cardiolipin molecules (orange lipid tails). The composition of the

other lipids (grey tails) is listed in Table II. The lipid PO₄ groups are dark green, the lipid choline or NH₃⁺ groups are colored magenta, and water molecules are blue.

FIG. 3.

Double-logarithmic plot of the mean squared displacement (MSD) for oxygen diffusion as a function of time. The average is taken over the 4 production runs and the 10 oxygen molecules. Transition counts are accumulated for all time intervals of length t along the trajectories.²² MSD in three dimensions in water and hexadecane, and MSD in radial direction parallel to the bilayer surface (x and y) in the POPC and MITO systems. The dashed red lines are linear fits $b + at$ to the MSD at long times, the dashed black line is the respective at line. Whereas a slope less than one in this double logarithmic representation

might be interpreted as subdiffusion, we emphasize that at short times, the apparent diffusion is substantially faster than that at long times. This behavior is consistent with cage diffusion, which is compensated for by the addition of an offset b in the fit $b + at$ to the MSD.

FIG. 4.

Free energy profile $F(z)$ for O_2 across the hexadecane/water slab (HEXD/WAT as specified in Table I): from Bayesian analysis, with Bayesian analysis error bars (blue), and from histogram counts (red, dashed line). The center of the hexadecane layer is located at $z = 0$ and the interface to water at $z \approx \pm 12$ $\text{\AA}$. Profiles are shifted vertically to set the minimum free energy as zero.

FIG. 5.

Diffusion profiles for O_2 across hexadecane/water slab (HEXD/WAT as specified in Table I) from Bayesian analysis: normal diffusion (a), radial diffusion (b), and anisotropy ratio $D_{\parallel}/D_{\perp}$ (c). The center of the bilayer is located at $z = 0$. Horizontal lines indicate the diffusion coefficients in neat water and neat hexadecane.

FIG. 6.

Free energy (a), anisotropy (b), normal diffusion (c), and radial diffusion (d) profiles for O_2 through the POPC and MITO membrane, from Bayesian analysis. The anisotropy is the ratio $D_{||}/D_{\perp}$. The center of the bilayer is located at $z = 0$.

FIG. 7.

Compartmental model for oxygen transport through membranes. Three regions are distinguished: the head group region $15 < |z| < 25$ Å (region 1), the lipid tail region $2.5 < |z| < 15$ Å (region 2), and the midplane region $|z| < 2.5$ Å (region 3). See Table IV for numerical values of the models.

FIG. 8.

Evolution of O_2 probability in POPC (left) and mitochondrial (MITO, right) membranes as a function of the traveled radial distance and the z -location in the membrane for lag times of 20 ps, 200 ps, 500 ps, 1 ns, and 5 ns. The plotted value is $\tilde{p}$ from Eq. 28 multiplied by 1000, based on profiles from Fig. 6. The initial position of the O_2 is the border of the membrane, $-25.5 \text{ \AA}$ and $-26.1 \text{ \AA}$ from the center of the membrane for the POPC and MITO membrane, respectively. The F and D profiles were extended with 100 additional bins (about $70 \text{ \AA}$) with

bulk water properties in order to reduce the effect of the z periodic boundary. The radial distance where the probability becomes zero was set to 150 Å.

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TABLE I.

Systems. Lipid composition in system MITO is presented in Tab. II

system	abbreviation	composition	$a = b$ [Å]	c [Å]	atoms
neat water	WAT	4328 water, 10 O ₂	50.	52.204	13004
neat hexadecane	HEXD	252 hexadecane, 10 O ₂	50.	51.433	12620
hexadecane/water	HEXD/WAT	126 hexadecane, 2159 water, 10 O ₂	50.	53.198	12797
POPC bilayer	POPC	72 POPC, 2242 water, 10 O ₂	48.082	67.925	16364
mitochondrial bilayer	MITO	72 lipids, 2890 water, 32 K ⁺ , 8 Cl ⁻ , 10 O ₂	52.425	69.569	19458

TABLE II.

Lipid composition of the simple membrane (POPC) and the inner mitochondrial membrane (MITO). The head groups are PC, PE, or CL. The tails *sn1* and *sn2* of the phospholipids are fatty acids $Cn:n'$, where n is the number of carbons in the tail and n' is the number of unsaturated bonds. TLCL has four tails, *sn1*, *sn2*, *sn1b*, and *sn2b*.

system	lipid name	lipids per leaflet	head	<i>sn1</i>	<i>sn2</i>	<i>sn1b</i>	<i>sn2b</i>
POPC	POPC	36	PC	C16:0	C18:2		
MITO	SAPE	9	PE	C18:0	C20:4		
	SLPE	9	PE	C18:0	C18:2		
	PLPC	12	PC	C16:0	C18:2		
	TLCL	6	CL	C18:2	C18:2	C18:2	C18:2

TABLE III.

Oxygen permeabilities of a layer of thickness h for the five studied systems. Normal permeability P is computed with Eq. 35, radial permeability $P_{\parallel}$ using Eq. 40, and average radial diffusion coefficient $D_{\parallel}^{\text{ave}}$ with Eq. 39. The HEXD/WAT and membrane values are based on the free energy and diffusion profiles from Figs. 4–6. The water and hexadecane values are based on $D = 0.603 \text{ \AA}^2/\text{ps}$ and $D = 0.455 \text{ \AA}^2/\text{ps}$, respectively (see Sec. III A).

system	h (Å)	P (cm/s)	$D_{\parallel}^{\text{ave}} (\text{\AA}^2/\text{ps})$	$P_{\parallel}$ (cm/s)	$P_{\parallel}/P$
POPC	51.6	26.	0.186	36.	1.38
MITO	52.9	36.	0.208	39.	1.09
WAT	52.1	116.	0.603	116.	1.
HEXD	52.1	87.	0.455	87.	1.
HEXD/WAT	52.1	277.	0.667	128.	0.46

TABLE IV.

Oxygen normal permeability P of a layer of $50 \text{ \AA}$ in the compartmental model (Eq. 38) of a lipid bilayer. Radial permeability $P_{\parallel}$ of a patch of this layer with length $50 \text{ \AA}$ in the x -direction (Eq. 41). Three regions are distinguished: the head group region $15 < |z| < 25 \text{ \AA}$ (region 1), the lipid tail region $2.5 < |z| < 15 \text{ \AA}$ (region 2), and the midplane region $|z| < 2.5 \text{ \AA}$ (region 3). Each model has a different set of diffusion constants D_i (in $\text{\AA}^2/\text{ps}$) and free energy differences F_i with the bulk water phase (in units $k_B T$), as depicted in Fig. 7. The normal and radial diffusivities are assumed to be equal in each compartment. Model **a** approximates the permeability of the POPC and MITO membranes.

model	D_1	D_2	D_3	F_1	F_2	F_3	$P \text{ (cm/s)}$	$P_{\parallel} \text{ (cm/s)}$	
a	0.15	0.1	0.3	1.	-2.	-3.	25.	34.	~ POPC, MITO
b	0.15	0.15	0.15	1.	-2.	-3.	26.	30.	no dip in D
c	0.15	0.1	0.3	0.	-2.	-3.	60.	34.	no F barrier
d	0.15	0.1	0.3	1.	0.	0.	16.	27.	no F well
e	0.15	0.1	0.3	0.	0.	0.	25.	28.	flat F profile