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Markovian and Non-Markovian Modeling of Membrane Dynamics with Milestoning

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Abstract

We exploit atomically detailed simulations and the Milestoning theory to extract coarse grained models of membrane kinetics and thermodynamics. Non-Markovian and Markovian theories for the phosphate group displacements are used to coarsely represent membrane motions. The construction of the two models makes it possible to examine their consistency and accuracy. The equilibrium and fluctuations of the phosphate groups along the normal to the membrane plane are estimated and Milestoning equations are constructed and solved. An optimal Markovian model is constructed that reproduces exactly the equilibrium and Mean First Passage Time (MFPT) of the Non-Markovian model. The equilibrium solution of both models is favorably compared to distributions obtained from straightforward Molecular Dynamics simulations. The picture for the kinetics is complex. Multiple local relaxation times of the mass density are illustrated emphasizing the non-Markovian characteristics of the process. In Markovian modeling only a single relaxation time is assumed for a state. Mapping of particle dynamics to the dynamics of a field density offers a new way of coarse graining complex systems as membranes that may bridge between atomically detailed models and phenomenological descriptions of macroscopic membranes.

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Introduction

Studies of dynamics of biological membranes have attracted a great body of experimental, computational, and theoretical work¹⁻⁹. This attraction is due to their biological significance. Membranes shape and surround living matter and present a barrier for passive transport of materials in and out of cells. Global fluctuations (micrometers) are relevant to dynamics of cells and cell compartments, and local membrane disruption (nanometers) can facilitate penetration of molecules in and out cells. Furthermore, temporal scales are also very large varying from nanoseconds for lipid diffusion in the membrane plane to minutes for phospholipid flips between membrane layers. It is therefore not surprising that modeling membranes at the atomic^{10,11} and at the mesoscopic scales¹² are prime topics of intense research. It is desirable to bridge and unify the atomic and mesoscopic descriptions of membranes having a single theory or computational methods that uses and provides information at multiple scales. However, the unification goal turned out to be difficult, and global and local dynamics of membranes are usually studied separately.

One promising direction that was already proven useful is of coarse graining of particles. Atomically detailed models are reduced to mechanical models with a smaller number of particles. This is achieved by grouping several atoms to a point mass and reducing the number of degrees of freedom that require detailed consideration. A successful and popular example of such a coarse grained model is the Martini force field^{13,14}. While this mapping of atomic point masses to a smaller number of point masses is successful, some questions remain. The “grouping approach” is consistent with direct calculation or modeling of a potential of mean force but it is incomplete for the study of kinetics. To study kinetics with a reduced set of variables compared to the full atomistic model we need more than an effective potential. It is necessary to estimate memory effects that are usually included in friction kernels such as in the theory of the Generalized Langevin Equation (GLE)¹⁵. The modeling or direct calculation of the memory function for a reduced set of variables is challenging¹⁶ and in practice the exact models are approximated by a Markov model with no memory. The friction kernel is reduced to a friction coefficient multiplying a Dirac delta function in the memory time. It is hard to assess these approximations since an exact solution for the friction kernel is rarely available.

Another approach to coarse graining kinetics is based on the Master and the Generalized Master Equations (GME)¹⁷. The Master Equation (ME) describes a Markov process, while the GME allows for non-Markov behavior by the inclusion of time dependent rate coefficient. The ME describes transitions between discrete states as a function of continuous time.

$$\frac{dP_{\alpha}}{dt} = \sum_{\beta} k_{\alpha\beta} P_{\beta} \quad (1)$$

where α or β label a state, t is the time, the probability of being in a state α is P_{α} and $k_{\alpha\beta}$ is the net rate coefficient for the transition between states α and β . In the present manuscript

we define a Markov process as a process with kinetics that follows the Master Equation (Eq. (1)).

The GME is taking a more general form

$$\frac{dP_{\alpha}(t)}{dt} = \sum_{\beta} \int_0^t \Gamma_{\alpha\beta}(t-t') \cdot P_{\beta}(t') dt' \quad (2)$$

which is reduced to Eq. (1) if we set $\Gamma_{\alpha\beta}(t) = k_{\alpha\beta} \cdot \delta(t)$

The challenge in solving Eq. (2) is similar in spirit to the challenge of solving the GLE. Computing the kernel of the Generalized Langevin Equation or the rate function $\Gamma(t)$ is far from trivial unless the process is Markovian. We are not aware of an efficient and exact algorithm to compute $\Gamma(t)$ from atomically detailed simulations.

About 12 years ago one of us introduced the method of Milestoning¹⁸. The Milestoning approach was introduced as an approximate method of computing system kinetic from short trajectories between milestones. Milestones were introduced as hypersurfaces normal to a reaction coordinate and more recently were extended to any hypersurfaces between cells determined by a set of coarse variables¹⁹ (Fig. 1). The short trajectories between milestones are used to estimate a transition kernel, $K_{\alpha\beta}(t)$, which is the probability that a trajectory that starts at milestone α at time zero will end up at milestone β exactly after time t . The kernel is

normalized as follows: $\sum_{\beta} \int_0^{\infty} K_{\alpha\beta}(t) dt = 1$. If the phase space points at the interface are also kept in the kernel (i.e. we have $K_{\alpha\beta}(x_{\alpha}, x_{\beta}, t)$ where x_{α} is a phase space point at interface α), then the algorithm is exact²⁰ for time homogeneous processes. The trajectory sampling in the exact version of Milestoning is equivalent to the non-equilibrium umbrella sampling of Dinner²¹ and trajectory tilting of vanden Eijnden.²² In the present manuscript we are interested however in the coarse graining and approximate procedure in which only information on the label of a milestone, α or β , is retained. The approximate theory leads to a much more efficient calculation. The procedure assumes that the phase space points at the interface, which are used to initiate trajectories between interfaces and estimate transition probabilities, are sampled from an equilibrium (canonical) distribution in the first iteration. An assumption of de-correlation or local equilibration must be satisfied. However, this assumption was shown to yield sound results many times.²³

Given the distribution of the initial conditions of trajectories at interface α at time zero, $P_{\alpha}(t=0)$, an equation was proposed to describe the time evolution of the system¹⁸:

$$q_{\alpha}(t) = P_{\alpha}(t=0) + \sum_{\beta} \int_0^t K_{\alpha\beta}(t-t') q_{\beta}(t') dt' \quad (3)$$

where $q_\alpha(t)$ is the absolute flux function. It is the number of trajectories that pass through milestone α (or enter state α) exactly at time t . The probability of a state α (the probability that the last milestone that was passed by a trajectory up to time t is α) is given by

$$P_\alpha(t) = \int_0^t q_\alpha(t') \left[1 - \int_0^{t-t'} K_{\alpha\beta}(\tau) d\tau \right] dt' \quad (4)$$

In the paper which introduces Milestoning¹⁸ we also showed in appendix B that Eq. (3) and (4) are equivalent to Eq. (2) or the Generalized Master Equation. The kernel in Eq. (3) and (4), $K_{\alpha\beta}(t)$, is computable, however, from straightforward short trajectories between milestones α and β as described in the Milestoning algorithm.²⁴ The direct computation of the kernel for a few coarse variables makes it possible to examine the Markov assumption for a non-trivial system. We have also shown²³ that for a Markov process that reproduces the same equilibrium distribution and the overall mean first passage time (MFPT) as the non-Markovian model we must have for the kernel

$$K_{\alpha\beta}^M(t) = \frac{N_{\alpha\beta}}{N_\alpha \langle t_\alpha \rangle} \exp\left(\frac{-t}{\langle t_\alpha \rangle}\right) \quad (5)$$

A superscript M denotes a Markov process, N_α is the total number of transitions starting from milestone α , $N_{\alpha\beta}$ is the number of these transitions that end in milestone β , and $\langle t_\alpha \rangle$ is the average life time of milestone α . Note that the kernel is normalized. The Markovian kernel is set in such a way that the equilibrium distribution and the mean first passage time are the same as for the more general non-Markovian process. Differences between the Markovian and non-Markovian behavior can be detected only in deviation of the second moment of the first passage time.

In the trajectory language the average milestone life time is estimated as follows: given a set of L trajectories that are initiated at milestone α at time zero, and integrated to termination at time t_i at other milestones β we average the termination times.

$$\langle t_\alpha \rangle = \frac{1}{L} \sum_{i=1, \dots, L} t_i \quad (6)$$

In a recent paper²⁵ we discussed an analysis of membrane and permeation dynamics using atomically detailed simulations and the theory of Milestoning. Milestoning translates particle dynamics to the dynamics and equilibrium of densities in coarse space. The coarsening made it possible to study processes of long times and spatial scales, for example, the translocation of water molecules through the membrane. In the present manuscript we extend our prior study to consider different models of kinetics based on Markovian and non-Markovian formulations.

These studies make it possible to examine widely used approaches for coarse graining such as Markov State Models^{26,27} in which Markovianity of the dynamics of coarse variable is assumed. By analyzing a coarse model that shows signatures of non-Markovian dynamics we are able to assess the importance of “time-memory” of membrane dynamics for different physical observables that we frequently compute. For example the overall mean first passage time, or the time evolution of the system. If we plug in the numerically computed kernel from atomically detailed trajectories to Eq. (3) and compute kinetic and thermodynamic behavior, we can compare it to the Markovian model of the kernel (Eq. (5)). This comparison is the focus of the present manuscript. We have chosen to study a membrane system since deviations from a Markov process for this system, which is important biologically, are visibly significant when we examine the time dependence of the kernels.

Theory of Milestoning for Fields

Milestoning for fields was introduced previously²⁵. The field concept is similar to earlier studies of hydrophobicity²⁸. In this section we will summarize and highlights the theoretical results of field modeling in the context of Milestoning.

We study the changes of the density $\rho_X(\mathbf{R})$ of a type of atoms X with Milestoning. This density represents the number of atoms in an infinitesimal volume element centered at $\mathbf{R}$.

As pointed out in the Introduction, the basic property examined by Milestoning is the flux, q , which we define as the number of trajectories that pass a milestone per unit time. In this work changes in the local density determines the milestone, therefore we have that the flux is expressed by a change of density between neighboring cells per unit time and at time t :

$$q(\Delta\rho(\mathbf{r}), t) = q(\rho(\mathbf{R}') \rightarrow \rho'(\mathbf{R}'), \rho(\mathbf{R}) \rightarrow \rho'(\mathbf{R}), t) \quad (7)$$

Where $\mathbf{r}$ is the position of the wall between two nearby cells centered at $\mathbf{R}$ and $\mathbf{R}'$ (Fig 1). During this transition cell $\mathbf{R}$ changes its density from $\rho(\mathbf{R})$ to $\rho'(\mathbf{R})$. This change of density can only happen if there is another change of density (of opposite sign) in a neighboring cell $\mathbf{R}'$ from $\rho(\mathbf{R}')$ to $\rho'(\mathbf{R}')$. We assume that all density changes are between two and not more cells.

To calculate the fluxes through different milestones we need to estimate the transition probability, $\mathbf{K}(\Delta\rho(\mathbf{r}), \Delta\rho'(\mathbf{r}'), t)$, that two sequential changes of density between neighboring cells will happen at a time interval t . This transition kernel is estimated from molecular dynamics trajectories. We can use a single long trajectory, which is chopped to many small fragments between milestones^{29,30}, or many short trajectories^{30–33} initiated on one milestone and terminated on another. From the trajectories we can determine instances in which the milestones $\Delta\rho(\mathbf{r})$ and $\Delta\rho'(\mathbf{r}')$ are passed in sequence. With the transition kernel we can solve the Milestoning equation for the flux:

$$q_X(\Delta\rho_X(\mathbf{r}),t)=p_X(\Delta\rho_X(\mathbf{r}),t)\cdot\delta(t-0^+)+\sum_{\mathbf{r}'}\int_0^t\int_{\Gamma_\rho}q_X(\Delta\rho'_X(\mathbf{r}'),t')K_X(\Delta\rho'_X(\mathbf{r}'),\Delta\rho_X(\mathbf{r}),t-t')dt'\cdot d(\Delta\rho'_X(\mathbf{r}')) \quad (8)$$

In the above formula we introduce $p_X(\rho_X(\mathbf{r}),t)$ which is the probability that the last milestone crossed at time t is $\Delta\rho_X$, and δ is the Dirac's delta function. The time integration in the second term on the right side of Eq. (8) considers all possible early passes of milestones at times $t' < t$ and the coarse space integration (Γ_ρ) is over all possible density changes in the pair of cells. In practical implementations of the algorithm we either consider the Laplace transform of Eq. (8) (see Eq. (9)) or more directly the densities are discretized and the time integral is replaced by a sum. The integral equation in time is solved numerically¹⁸

Eq. (8) (or Eq. (3)) can be solved with the help of Laplace transforms (the details of the derivation can be read somewhere else^{20,23,24,34}). The stationary flux vector (where the vector components are the stationary flux through each milestone) is computed as the solution of the linear equation

$$\mathbf{q}_{stat} = \langle \mathbf{q}_{stat} \mathbf{K} \rangle \quad (9)$$

The average corresponds to the integral over the phase space points of the trajectories that pass through the milestones. Cyclic boundary conditions, useful for calculations of stationary flux, are imposed. The transition matrix $\mathbf{K}$ is set to return trajectories that hit the final milestone back to the initial milestone. We also determine the average lifetime of a milestone, $(\mathbf{t})_\alpha \equiv t_\alpha$ which is the average time trajectories that are initiated at the same milestone (say α) are run before terminating on another milestone. In other words the vector $\mathbf{q}$ determines the initial conditions of the trajectories within the milestone. The operator $\mathbf{K}$ provides the outcomes of the Molecular Dynamics trajectories between the milestones.

The stationary probability vector of all milestones is given by

$$\mathbf{p} = \mathbf{q} \odot \mathbf{t} \quad (10)$$

where the symbol $\odot$ denotes element wise product and the vector $\mathbf{t}$ contains the life-time of every milestone in the system

$$(\mathbf{t})_{\Delta\rho_X(\mathbf{r})} = \sum_{\mathbf{r}'} \int_{\Gamma_\rho} \int_0^\infty t \cdot \mathbf{K}_X(\Delta\rho_X(\mathbf{r}), \Delta\rho_X(\mathbf{r}'), t) dt \cdot d(\Delta\rho_X(\mathbf{r}')) \quad (11)$$

where $(\mathbf{t})_{\Delta\rho_X(\mathbf{r})}$ is the average life time of milestone $\Delta\rho_X(\mathbf{r})$.

Finally, the overall mean first passage time is computed using²⁴

$$\langle \tau \rangle_f = \mathbf{p}_0 (\mathbf{I} - \overline{\mathbf{K}})^{-1} \mathbf{t} \quad (12)$$

where $\mathbf{p}_0$ is the initial probability that a milestone was crossed. The kernel $\overline{\mathbf{K}}$ is similar to $\mathbf{K}$ in Eq. (9) except that an absorbing boundary condition is used at the final milestone f to compute the Mean First Passage Time (MFPT). A different and more convenient expression, which was proved to be equivalent to Eq. (12), can be used to calculate the MFPT²⁰

$$\langle \tau \rangle_f = (\mathbf{q}^t \cdot \mathbf{t}) / q_f \quad (13)$$

where q_f is the flux through the final absorbing milestone.

Using a generating function it is possible to compute higher moments of the first passage time (FPT)²⁰. For example the second moment of the FPT is given by

$$\langle \tau^2 \rangle_f = (\mathbf{q}^t / q_f) \left[2\mathbf{T}(\mathbf{I} - \mathbf{K})\mathbf{t} + \mathbf{t}^{(2)} \right] \quad (14)$$

where

$$(\mathbf{T})^{(2)} = \int_0^\infty t^2 \mathbf{K}(t) dt \quad \mathbf{t}^{(2)} = \mathbf{T}^{(2)} \mathbf{1} \quad (15)$$

$\mathbf{1}$ is a vector of ones.

Methods

A. MD Simulations

The density data was retrieved from MD simulations we conducted in our previous work²⁵. The simulated bilayer membrane contains 128 DOPC (1,2-dioleoyl-sn-glycero-3-phosphocholine) molecules (64 in each leaflet), and 6097 water molecules. The initial configuration of the system was prepared using the membrane builder facility of CHARMM-GUI^{35,36}. The force fields used in the simulations were the united-atom Berger force field for the acyl chain atoms of the lipid³⁷ and OPLS force field³⁸ for the head group region. Water molecules were modeled using the TIP3P force field³⁹. This combination of Berger/OPLS parameters for the lipid and TIP3P model for water has been used by others^{40,41}. The MD simulations were conducted with our molecular package MOIL^{42,43}. After 2 ns of an equilibration run a production run of 200 ns was done at 300 K and constant volume (the box size is $66.0 \times 66.0 \times 74.9 \text{ \AA}^3$), with configurations saved every picosecond. The surface area per lipid is $68.1 \text{ \AA}^2$ that is in the same range obtained in previous simulations of the same phospholipid membrane⁴⁴ and 5 % smaller than the experimental value⁴⁵.

We used a 1 fs time step to integrate the bonding, van der Waals and real part of the electrostatics interactions and a 4 fs time step to evaluate the reciprocal-space component of the Ewald sum using the multiple time stepping algorithm r-RESPA⁴⁶. We used a matrix implementation of the SHAKE algorithm^{47,48} to constrain water bond length and angles. The long-range electrostatic forces were calculated using the smooth particle mesh Ewald method⁴⁹ with a grid of $64 \times 64 \times 76$.

B. Density representation

To extract number density information for the location of the phosphate groups from the MD simulations we mapped the simulation box onto a three-dimensional rectangular grid (Fig. 2). The number of cubic grids used for this mapping was $15 \times 15 \times 17$, with cell size $4.4 \text{ \AA}^3$. The number of phosphate atoms (including both phosphorous and oxygen atoms) in every grid was counted and the density of the grid was classified according to this number of atoms. To determine the current milestone of a cubic grid we count the number of atoms in the grid. We group the number of atoms in four sets to improve the statistics of the local transitions. The “zero” density milestone contains zero or one phosphate atom, the low density milestone contains two or three phosphate atoms, the middle density milestone contains four or five atoms and the high density milestone contains six or more atoms.

C. Computation of transitions of density

The algorithmic part is similar to what was described in the previous paper²⁵. To explore transitions we keep track of the densities in neighboring cells as a function of time. There are 26 neighbors to a central cell. We assume symmetry in the membrane plane that reduces the number of distinct nearby cells to only 8 (see Figure 3 of reference²⁵). To describe a transition (Fig. 1), we need to consider two sequential elementary events at cell R' to build the transition kernel. The first event is of density transfer between the pair of cells (R, R') and the second event transfers density between cells (R', R'') after time t . We estimate the transition kernel for the type of atoms X as $K_{XRR',R'R''}(t) \cong n_{RR',R'R''}(t)/n_{RR'}(0)$, where $n_{RR'}(0)$ is the number of (R, R') events initiated at time zero, and $n_{RR',R'R''}(t)$ is the number of trajectories initiated at (R, R') at time zero that continue to event (R', R'') exactly at time t .

In the present discretization with cubic cells the number of milestones is $M=(8 \times 17 \times 4 \times 4)$ where 8 is the number of distinct cells surrounding a central cell²⁵, 17 is the number of grids along the membrane axis and 4 is the number of density labels used to count the number of phosphate atoms in each cell. In the current analysis we used only the 4 density labels defined earlier for the phosphate atom (zero, low, middle, and high density). Because our interest is focused on the diffusion along the membrane axis we grouped transition from the top three cells and added them to the cell at the top of the cell of interest. A similar grouping was done for the cells at the bottom and the lateral cells. These combinations reduce the calculation to transitions in a single column along the membrane axis. This simplification reduces the number of possible milestones to $M=(3 \times 17 \times 4 \times 4)=714$. Because the phosphates are rarely located at the membrane

center or at the water phase (Fig. 2) the number of active milestones that were actually visited during the MD simulation was smaller (90).

Results and Discussion

The mass transfers between the cells during the MD trajectory described earlier were used to extract information about transitions of density between milestones. Table 1 illustrates the number of transitions between milestones, probabilities of transitions and milestone life times for milestones located in one of the two leaflets of the membrane. Transitions between several milestone pairs are observed very often during the simulation with a larger number occurring for milestones located in between 22.0 to 13.2 Å from the membrane center. It is noted that for most locations along the membrane axis (the z axis) the number of transitions to the high density milestones tends to be lower compared to transitions involving the zero, low and middle density milestones. For this reason most of the results shown in this section do not involve transitions to or from the high density cells. The average milestone lifetime tend to be short with the larger lifetimes occurring for transition from the zero density cells.

Using Milestoning Eq. (10) we computed the stationary probability distribution to observe phosphate densities at the different milestones that we defined in the Method section. The results are shown in Fig. 3 where they are also compared with the probabilities extracted directly from the MD simulation data. The Milestoning results are close to the values extracted from the original data. Outside the headgroup region of the membrane (around 18 Å away from the membrane center) the probability of finding phosphate atoms is very low and this is the reason the zero density milestone is the most populated everywhere even at the headgroup region. Low density and middle density milestones are visited in the headgroup region of the membrane but the high density milestones are less populated. The agreement between the Milestoning results and the original MD data suggests that Milestoning describes correctly the equilibrium properties of the diffusive motion of the phosphate atoms in the direction normal to the membrane plane and it gives us confidence that additional rate information extracted from the calculations should be reliable.

In Fig. 4 we show the time dependence of local transition probabilities between milestones with zero, low and middle densities at four different locations along the membrane axis. The transition data shows complex behavior that cannot be captured by a single exponential decay. For example, in Fig. 4a depicting transitions from zero to low density, the decay corresponding to density transitions from a milestone at 17.6 to 13.2 Å is fitted by three exponential functions (with exponential arguments of 0.37, 0.098, and 0.018 ps⁻¹). In other words, this milestone lacks the meta-stability property that is required to define a locally thermal “state”. Since our Milestoning analysis is based on the fluxes and not on metastability of a population within a cell such multiple relaxation times can be captured by the non-Markovian kernel.

For the rest of the transitions shown in Fig. 4 it is also clear that a single exponential function does not fit the data accurately. Although the exact origin of these different time scales is difficult to determine from our analysis (we do not distinguish, for example between intramolecular or intermolecular phospholipids motions), the faster motions are

likely to be associated with small up and down displacements of a phosphate group of a single phospholipid molecule. The slower motions could be associated with more collective diffusion involving two or more phospholipids that during their lateral motion can produce changes of the phosphate density of neighboring cells.

Fig. 5 shows the time dependence of local flux between milestones for two different locations along the membrane axis. These plots are obtained by solving Milestoning Eq. (8) for the flux using time dependent transition probability $K_{\alpha\beta}(t)$ (some of them plotted in Fig. 4). We also calculated the flux assuming a Markovian model for the kernel, or an exponential transition probability (Eq. (5)), with the decay rates being the inverses of the milestone life times (given in Table 1). Some quantitative differences are observed for the fluxes of the Markovian and non-Markovian approaches.

As we argued earlier, the equilibrium properties and a measure of the first order kinetics (or the stationary transition matrix $K_{ij} \equiv \int_0^\infty K_{ij}(t)dt$ and the mean first passage time) are the same in the non-Markovian model and the Markovian model of our design. Differences can be detected only at averages of higher moments of the passage time. A measure of non-Markovian behavior is provided by the average of the second moment of the transition times.

Table 2 shows a comparison between these second order moments in time extracted using Milestoning analysis and the ones computed using Markovian assumptions (that gives $\langle t_\alpha^2 \rangle = 2\langle t_\alpha \rangle^2$). For some milestones the difference between the Markovian and Non-Markovian estimates is small but for a few of them the difference is large (notably for zero density milestones). In Fig. 4 we showed the time dependence of $K_{\alpha\beta}(t)$ and the largest deviation from exponential behavior occurs at transitions starting from zero density.

Do these deviations from Markovian behavior detected for local properties (transitions between cells) influence significantly global measures and overall kinetics? We estimated the overall kinetics of a phosphate density fluctuation at $z = 30.8 \text{ \AA}$ (from zero to low density) and how long it takes before an opposite phosphate density variation (from low to zero density) occurs in a region of the membrane closer to the center at $z = 8.8 \text{ \AA}$. To ensure the significance of this comparison we assess the error bars in the calculations using a statistical model that we developed earlier.²⁹ In brief, the model is set as follows: A transition matrix element, $K_{\alpha\beta}$, is estimated from an ensemble of trajectories initiated at milestone α . Each of the trajectories yields a weight of 0 or 1 for the transition. It either ends at milestone β with a weight of one or has a weight of zero if it ends someplace else. The normalized transition probability estimated from L trajectories is simply $K_{\alpha\beta} = (1/L)(1+0+0+\dots+1+\dots+1)$ where L is the number of trajectories. For a large number of uncorrelated events for which the average and the variance are finite the Central Limit Theorem holds and $K_{\alpha\beta}$ is sampled from a normal distribution with mean and variance extracted from the simulation data. We sampled individual $K_{\alpha\beta}$ from this normal distribution (with a sampling of 1000 values) while enforcing the condition: $\sum_\beta K_{\alpha\beta} = 1$ by

renormalization of the sampled elements. A similar procedure was followed to sample the average lifetime and its second moment in time. This statistics was used to compute a distribution of stationary fluxes, of MFPTs and of second moments of the passage times and to estimate uncertainties. For the density change described previously the overall MFPT is $(1.82 \pm 0.17) \times 10^6 \text{ ps}$ and its second moment is $(4.54 \pm 0.82) \times 10^{13} \text{ ps}^2$. The Markovian estimate for this second moment is $(6.72 \pm 1.22) \times 10^{12} \text{ ps}^2$. There is almost a one order of magnitude difference between the two values of the second moment. Hence the difference is significantly larger than the numerical uncertainties.

Conclusions

We outlined a probabilistic reduced model for the fluctuations of phospholipid mass in a biological membrane. It is based on the Milestoning theory for fluxes. In Milestoning a Non-Markovian model is constructed for a selected subset of coarse variables which in the present case are the phosphate group mass densities. The model is rigorously derived from atomically detailed simulations²⁵. It can be used as a bridge between continuum theories, for example the description of the membrane as viscous fluid and atomistic models.⁵⁰.

The present study also explore the use of Markovian and Non-Markovian modeling. A widely used model for coarse graining is the Markov State Model.^{26,27} It is not easy to assess the accuracy these models for complex systems. Milestoning that can be formulated and solved with a Markovian and non-Markovian expressions makes this comparison for large molecular systems possible. We investigated membrane dynamics using a Milestoning approach based on Markovian model as outlined in²³ and in⁵¹ and non-Markovian Milestoning.

In addition to the direct comparison we outlined a simple test of local Markovianity (the exponential decay in time of the transition kernel) and found significant numerical deviation between the two models. Besides local deviations, macroscopic differences are observed for the second moment of the first passage times. The moments differ by about an order of magnitude. Keeping these significant errors in mind, it is useful to examine simulation data of trajectory fragments between states and verify Markovianity before constructing Markovian models.

Our results add to and quantify the experimental and theoretical evidence illustrating that the membrane dynamics is extremely diverse in interactions and time scales. Future research will determine the different molecular mechanisms that are involved in the complex diffusion observed for the phosphate headgroups of the membrane.

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Figure 1.

Transition of density changes between two milestones. In this representation cells are separated by walls. For example, the transition we consider is between the gray walls at $\mathbf{r}$ and $\mathbf{r}'$. The density $\rho(\mathbf{R})$ is the number of atoms of interest (in this work, the phosphate atoms) in the cell centered at $\mathbf{R}$. We consider transitions of density between two different boundaries belonging to the same cell, in this case cell $\mathbf{R}'$. A transition event starts with a change of densities of the cells separated by $\mathbf{r}$: $\Delta\rho(\mathbf{r})=\rho(\mathbf{R}') \rightarrow \rho'(\mathbf{R}')$, $\rho(\mathbf{R}) \rightarrow \rho'(\mathbf{R})$ (pictorially, that change occurs when the cyan trajectory line crosses wall $\mathbf{r}$). We set the time of this density change to zero since the time origin is not important for transition probabilities in time homogeneous processes. The densities $\rho'(\mathbf{R})$ and $\rho'(\mathbf{R}')$ are the densities of those two cells at $t=0$. The density of $\mathbf{R}'$ is followed until the next transfer of mass occurs through a different wall $\mathbf{r}'$ at time t : $\Delta\rho'(\mathbf{r}')=\rho'(\mathbf{R}') \rightarrow \rho''(\mathbf{R}'')$, $\rho'(\mathbf{R}''') \rightarrow \rho''(\mathbf{R}'')$ (this change occurs when the purple line trajectory crosses wall $\mathbf{r}'$). The transition kernel $\mathbf{K}(\Delta\rho(\mathbf{r}), \Delta\rho'(\mathbf{r}'), t)$ is the probability that the density change $\Delta\rho(\mathbf{r})$ at wall $\mathbf{r}$ is followed by the density change $\Delta\rho'(\mathbf{r}')$ at wall $\mathbf{r}'$ after time t . For brevity we also say that the transition occurs from milestone $\Delta\rho(\mathbf{r})$ to milestone $\Delta\rho'(\mathbf{r}')$ where the term milestone refers both to the location of the wall and the density change associated with it.

Figure 2.

A molecular snapshot of the membrane bilayer system (a) mapped into a three-dimensional cubic grid (b). The phosphate atoms, that are the focus of the present work, are represented as large van der Waals spheres, while the membrane and water atoms are represented as CPK models. The size of the cubic grids displayed in (b) matches the size used in the analysis of the density fluctuations of the phosphate atoms.

Figure 3.

Equilibrium probabilities to find the system at different milestones for phosphate density along the membrane axis. The solid lines are the results of binning a long molecular dynamics trajectory and the dashed lines are the Milestoning results, computing the flux and the probabilities (Eq. (9) and (10)). The center of the bilayer is at 0 Å. The headgroup region of the membrane where most phosphate atoms are located (see also Fig. 2) is located at about ± 18 Å.

Figure 4.

Transition probability densities as a function of time for transitions between milestones from zero to low density (a), from low to zero density (b), from low to middle density (c), and from middle density to low density (d). For all these transitions the terminating milestone is closer to the membrane center than the initial milestone. Transition data was extracted from density changes in both layers of the membrane. In a few cases (panel a, for example) transitions still occur after 100 ps but they are less frequent and the statistics are poor.

Figure 5.

Time dependence of the flux at two different locations along the membrane axis. Panel a shows transitions from $z = 22.0 \text{ \AA}$ to $17.6 \text{ \AA}$ with the initial seed for the flux calculation placed at milestones located at $z = 26.4 \text{ \AA}$. Panel b shows transitions from $z = 17.6$ to $13.2 \text{ \AA}$ with the initial seed located at $22.0 \text{ \AA}$. The plots show comparison between the fluxes of non-Markovian (solid lines) and Markovian (dashed lines) fluxes.

Table 1

Milestone transition data for positive z layer extracted from the MD simulations. The first two columns show the position and density of the initial milestone. The third and forth columns show the position and density of the terminating milestone. The fifth column is the number of transitions. The sixth column provides the transition probability between the milestone, and the last column the lifetime of the initial milestone. Transitions starting from high density milestone are not shown (typically only tens of those transitions were observed during the analysis of the simulation data). Due to their rarity and poor statistics, transitions of more than 500 picoseconds were not use in the averages.

Initial z (Å)	Initial density	Final z (Å)	Final density	# of transitions	K _{ij}	Milestone life times (ps)
26.4	Zero	22.0	low	6211	1	27.6
26.4	Low	22.0	zero	3600	0.4126	1.96
26.4	Low	22.0	middle	5126	0.5874	1.96
26.4	Middle	22.0	low	1407	0.9915	1.94
26.4	Middle	22.0	high	12	0.0085	1.94
22.0	Zero	17.6	low	199199	0.9853	36.1
22.0	Zero	26.4	low	2965	0.0147	36.1
22.0	Low	17.6	zero	217510	0.5765	2.35
22.0	Low	17.6	middle	140122	0.3714	2.35
22.0	Low	26.4	zero	18025	0.0478	2.35
22.0	Low	26.4	middle	1648	0.0043	2.35
22.0	Middle	17.6	low	76054	0.9416	2.70
22.0	Middle	17.6	high	234	0.0029	2.70
22.0	Middle	26.4	low	4485	0.0555	2.70
17.6	Zero	13.2	low	160011	0.5217	15.9
17.6	Zero	22.0	low	146679	0.4783	15.9
17.6	Low	13.2	zero	526546	0.4703	2.61
17.6	Low	13.2	middle	98807	0.0883	2.61
17.6	Low	22.0	zero	408439	0.3648	2.61
17.6	Low	22.0	middle	85711	0.0766	2.61
17.6	Middle	13.2	low	133663	0.5183	3.57
17.6	Middle	13.2	high	66	0.0002	3.57
17.6	Middle	22.0	low	124102	0.4812	3.57

Initial z (Å)	Initial density	Final z (Å)	Final density	# of transitions	K _{ij}	Milestone life times (ps)
17.6	Middle	22.0	high	72	0.0003	3.57
13.2	Zero	8.8	low	513	0.0022	33.6
13.2	Zero	17.6	low	230537	0.9978	33.6
13.2	Low	8.8	zero	11183	0.0294	2.35
13.2	Low	8.8	middle	254	0.0006	2.35
13.2	Low	17.6	zero	219493	0.5768	2.35
13.2	Low	17.6	middle	149623	0.3932	2.35
13.2	Middle	8.8	low	1394	0.0153	2.84
13.2	Middle	17.6	low	89633	0.9817	2.84
13.2	Middle	17.6	high	273	0.0030	2.84
8.8	Zero	13.2	low	1958	1	27.7
8.8	Low	13.2	zero	594	0.2698	1.88
8.8	Low	13.2	middle	1608	0.7302	1.88
8.8	Middle	13.2	low	237	0.9834	1.69
8.8	Middle	13.2	high	8	0.0166	1.69

Table 2

Second order moments of Milestoning lifetime extracted from a Milestoning analysis of the membrane data is compared to the estimates obtained using Markovian approach. The results are for the positive z layer of the bilayer membrane.

Location along z axis (Å)	density	Markovian $\langle t_i^2 \rangle$, ps ²	Non-Markovian $\langle t_i^2 \rangle$, ps ²
26.4	zero	1528.9	10843.
26.4	low	7.673	7.249
26.4	middle	7.546	9.185
22.0	zero	2605.2	16232.
22.0	low	11.086	11.843
22.0	middle	14.540	24.085
17.6	zero	503.04	3766.4
17.6	low	13.643	14.786
17.6	middle	25.514	51.049
13.2	zero	2260.1	15041.
13.2	low	11.024	11.995
13.2	middle	16.159	32.350
8.8	zero	1532.8	11879.
8.8	low	7.044	6.665
8.8	middle	5.692	4.327