

THE UNIVERSITY OF CHICAGO

STATISTICAL MECHANICAL AND TRANSITION PATH THEORY APPROACHES
TO MEMBRANE PERMEATION

A DISSERTATION SUBMITTED TO
THE FACULTY OF THE DIVISION OF THE PHYSICAL SCIENCES
IN CANDIDACY FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

DEPARTMENT OF CHEMISTRY

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CHICAGO, ILLINOIS
AUGUST 2026

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To my wife.

TABLE OF CONTENTS

LIST OF FIGURES	viii
LIST OF TABLES	xii
ACKNOWLEDGMENTS	xiii
ABSTRACT	xiv
1 INTRODUCTION	1
1.1 Biological membrane permeability	1
1.2 Statistical mechanics and transition path theory	3
1.2.1 Brownian dynamics and the ISD model	3
1.2.2 The generalized Langevin equation	5
1.2.3 The effect of memory on the rate of barrier crossing	9
1.3 Comparing simulations with experiment	10
2 STATISTICAL MECHANICAL THEORIES OF MEMBRANE PERMEABILITY	14
2.1 Introduction	15
2.2 Theoretical Developments	17
2.2.1 Membrane permeability and Green-Kubo linear response	17
2.2.2 Net flux, recrossings, and transmission coefficient	22
2.2.3 Counting membrane crossings from reactive trajectories	23
2.2.4 Transition path theory, committor probability, and permeability	25
2.2.5 Generalized Langevin equation and correction to the ISD model	28
2.3 Simulations and Methods	32
2.3.1 Simulation of the overdamped GLE	32
2.3.2 Water permeation	34
2.4 Results and discussion	36
2.4.1 MSD on a flat free-energy landscape	36
2.4.2 Grote-Hynes correction to the ISD permeability	37
2.4.3 Permeability from BD simulations with memory effects	39
2.4.4 Water permeability	45
2.5 Concluding Discussion	47
3 MEMBRANE PERMEABILITY OF SUCROSE CALCULATED FROM EQUILIBRIUM TIME-CORRELATION FUNCTIONS USING MOLECULAR DYNAMICS SIMULATIONS WITH ENHANCED SAMPLING	51
3.1 Introduction	52
3.2 Theoretical developments	55
3.2.1 Inhomogeneous solubility-diffusion (ISD)	55
3.2.2 Time-correlation formulation of membrane permeability	56
3.2.3 Evaluation of the time-correlation functions	58

3.3	Simulations and Methods	62
3.3.1	Numerical validation from stochastic simulations	62
3.3.2	Optimal sampling with short trajectories and variance reduction	65
3.3.3	All-atom MD simulation protocol	68
3.3.4	Enhanced sampling simulations	68
3.4	Results and discussion	70
3.4.1	Validation of computational framework with BD stochastic simulations	70
3.4.2	Membrane permeability of sucrose from enhanced-sampling simulations	71
3.4.3	Comparison with experimental measurements	77
3.5	Concluding Discussion	80
4	DEEP LEARNING OF POSITION-DEPENDENT DIFFUSIVITY FROM UMBRELLA SAMPLING MOLECULAR DYNAMICS SIMULATIONS	82
4.1	Introduction	82
4.2	Theoretical developments	85
4.2.1	Multilayer perceptron (MLP) position-dependent diffusivity	86
4.2.2	Position-dependent diffusivity	88
4.3	Computational details	90
4.3.1	Molecular dynamics simulations	90
4.3.2	Numerical simulation of the GLE	91
4.3.3	Determination of the diffusivity	95
4.4	Results	97
4.4.1	Extraction of the position-dependent memory function with the MLP	97
4.4.2	Validation of the numerical integration of the GLE	101
4.4.3	Determination of the position-dependent diffusivity	103
4.4.4	Evaluation of the permeability	109
4.5	Conclusion	113
4.6	Appendix: Derivation of the Woolf-Roux and Hummer diffusivity	115
5	SYSTEMATIC SHORT UNBIASED TRAJECTORY SIMULATION WITH GEN- ERALIZED LANGEVIN AUTOMATED KINETIC ENHANCED SAMPLING (GLAKES)	117
5.1	Introduction	117
5.2	Theoretical developments	117
5.2.1	Estimating permeability via generalized Langevin automated kinetic enhanced sampling (gLakes)	117
5.3	Implementation details	119
5.4	Results	122
5.4.1	Extraction of the permeability coefficient with gLakes	122
5.5	Conclusion	125
6	HOW IS MEMBRANE PERMEATION OF SMALL IONIZABLE MOLECULES AF- FECTED BY PROTONATION KINETICS?	126
6.1	Introduction	127

6.2	Theoretical Developments	132
6.2.1	Equilibrium and kinetics of ionizable states	132
6.2.2	Spatial diffusion and discrete ionization	133
6.2.3	TPT formulation of the net reactive flux	138
6.3	Simulations and Methods	140
6.3.1	WTM-eABF computations	140
6.4	Results and discussion	143
6.4.1	Potential of mean force and position-dependent diffusivity	143
6.4.2	Comparison of pH-partitioning and Boltzmann-weighted average potential	146
6.4.3	Influence of ionization kinetics	150
6.4.4	Comparison with experiments and previous calculations	157
6.5	Conclusions	162
7	PERMEABILITY OF PROTONATED NICOTINE	164
7.1	Introduction	164
7.2	Theoretical developments	166
7.2.1	Reactive flux formulation of permeability	166
7.2.2	Enhanced sampling of auxiliary collective variable at the top of the barrier	168
7.3	Simulations and Methods	169
7.3.1	The mean-force string method of collective variables	169
7.3.2	PMF calculations	171
7.3.3	Short unbiased trajectories for transmission coefficient permeability calculation	171
7.4	Results and discussion	172
7.4.1	Re-weighted transmission coefficient permeability	172
7.5	Concluding discussion	178
8	CONCLUSION	179
APPENDIX A SUPPORTING INFORMATION FOR CHAPTER 2		181
A.1	Derivation of analytical MSD in overdamped GLE with exponential memory and harmonic potential	181
A.2	Derivation of Grote-Hynes correction to ISD permeability	186
A.3	Heaviside correlation function permeability from BD simulations with and without memory.	188
A.4	Position-dependent diffusivity of water in DLPC from umbrella sampling.	189
APPENDIX B SUPPORTING INFORMATION FOR CHAPTER 3		192
B.1	Statistical analysis of equilibrium and short unbiased 2D-Brownian dynamics trajectories	192
B.2	Committer, distributions of initial positions, and error analysis of short unbiased MD simulations of sucrose permeation	194

B.3	Position-dependent diffusivity	197
B.4	Hydration number of sucrose	198
APPENDIX C SUPPORTING INFORMATION FOR CHAPTER 4		199
C.1	Multilayer perceptron (MLP) loss function comparison	199
C.2	Comparison of integration schemes for numerical generalized Langevin simulations	201
C.3	Multilayer perceptron (MLP) structure	202
C.4	Time-correlation and memory functions (MD)	203
C.5	Woelf-Roux diffusivity extrapolation (MD)	206
C.6	Hummer diffusivity position autocorrelation function integrals (MD)	209
C.7	Woelf-Roux diffusivity extrapolation (GLE)	210
C.8	Hummer diffusivity position autocorrelation function integrals (GLE)	213
C.9	Time-correlation functions (GLE)	214
C.10	Grote-Hynes analysis of the MD $z = 0$ umbrella window	216
APPENDIX D SUPPORTING INFORMATION FOR CHAPTER 6		219
D.1	Theory	219
D.2	Enhanced sampling convergence	221
D.3	Lag time dependence of diffusivity	222
D.3.1	MSD diffusivity	222
D.3.2	Time-fractional Smoluchowski model	223
D.3.3	Investigation of ionization state dependence of the diffusivity profile	225
D.4	Boltzmann-weighted average diffusivity	227
D.5	Derivation of empirical relationship between protonated and neutral state contributions to pH-dependent permeability	228
REFERENCES		231

LIST OF FIGURES

1.1	The normalized time-dependent concentration of molecules with varying permeability coefficients inside a hypothetical LUV with a radius of 50 nm.	12
2.1	Physical situation of a membrane separating a bulk region a and a bulk region b containing a permeant molecule.	18
2.2	Brownian dynamics with exponential memory trajectory with color-coded crossings.	24
2.3	MSD diffusivity computed by Brownian dynamics with exponential memory simulations on a flat free energy landscape with various values of K_e and D_e	35
2.4	Effective MSD diffusivity as a function of the lag time computed analytically from eq 2.26 with an exponential memory for different barriers.	37
2.5	PMF analytical MSD diffusivity for demonstrating the Grote-Hynes correction to the ISD permeability of an inverted harmonic barrier with exponential memory.	38
2.6	PMF and approximate committor for the barriers used in the BD_e and BD simulations.	40
2.7	Total number of crossing events from BD_e and BD simulations of each barrier.	41
2.8	Time-correlation functions and permeability compared with counting and the ISD model for Brownian dynamics with exponential memory simulations.	43
2.9	PMF, committor, time-correlation functions, and permeability of water across a DLPC membrane, from MD simulations of the Drude force field.	45
2.10	Trajectories of water crossing a DLPC membrane from equilibrium MD simulations.	48
3.1	Results of BD stochastic simulations with non-Markovian memory effects.	72
3.2	PMF and position-dependent diffusivity of sucrose across DOPC and DLPC membranes with the C36 additive and Drude2023 polarizable force fields	73
3.3	Time-correlation functions and permeability of sucrose across DOPC and DLPC membranes from MD simulations of the C36 force field.	74
3.4	Time-correlation functions and permeability of sucrose across DOPC and DLPC membranes from MD simulations of the Drude force field.	75
4.1	Optimized memory functions extracted from umbrella sampling MD simulations of water in a membrane	98
4.2	The position-dependent parameters of the optimized memory function extracted by the MLP from umbrella sampling MD simulations of water in a membrane.	99
4.3	Assessment of the EM-3m numerical integration scheme for the GLE with the position-dependent memory function and the PMF of water extracted from MD simulations of water across a DLPC membrane.	102
4.4	Position-dependent diffusivity from umbrella sampling MD simulations of water across a DLPC membrane computed with Woolf-Roux, Hummer, and MLP approaches.	104
4.5	Examining the accuracy of the relationship between the Laplace transforms of the velocity and position autocorrelation functions.	106
4.6	Position-dependent diffusivity from umbrella sampling numerical GLE simulations with the EM-3m integrator.	108

4.7	All of the reactive trajectories from the equilibrium numerical GLE simulations.	110
5.1	Results of the gLakes simulations for the coarse-grained water-membrane system.	124
6.1	Chemical structures and Henderson–Hasselbalch fractions of each protonation state of nicotine and varenicline	128
6.2	PMF and position-dependent diffusivity for each protonation state of nicotine and varenicline.	144
6.3	PMFs of each protonation state of nicotine and varenicline, offset by the free energy of protonation in bulk solution for several pHs.	147
6.4	Effective pH-dependent permeability based on the BWAP and pH-partitioning treatments for nicotine and varenicline.	148
6.5	Committors of each protonation state across the membrane based and the components δJ_{off} and δJ_{on} of the deprotonation/protonation contribution to the overall steady-state flux for nicotine and varenicline.	153
6.6	Logarithm of permeability versus pH for each component of the total reactive flux and ratio of the permeability derived from the BWAP and perturbed reactive flux to the pH-partitioning treatment for nicotine and varenicline.	154
6.7	Typical permeation trajectories of nicotine generated by combining Brownian dynamics with Markov state model.	156
7.1	Illustration of the four collective variables used for optimizing the pathway with the mean force string method and the converged string.	173
7.2	PMFs of the primary and auxiliary collective variables of protonated nicotine across the POPC membrane.	174
7.3	Scatter plots of the 2-dimensional coordinates for each short unbiased trajectory for $t = 0$ and $t = 500$ ps.	176
7.4	The permeability of protonated nicotine based on the re-weighted transmission coefficient, the re-weighted indicator restricted committor transmission coefficient, and the ISD model.	177
A.1	Permeability computed with the Heaviside time-correlation function for BD (top) and BD_e simulations.	188
A.2	Umbrella window probability density for water in DLPC with a harmonic force constant of $10 \text{ kcal/mol } \text{\AA}^2$ and centers spaced at $1 \text{ \AA}$ intervals.	189
A.3	Position-dependent diffusivity profile of water in a DLPC membrane, over a range of upper limits of the integral of the position autocorrelation function.	190
A.4	Position-dependent diffusivity profile of water in DLPC, under the condition that t_∞ satisfies $C_{zz}(t_\infty) < 10^{-5} \text{ var}(z)$ or else $t_\infty = 500$ ps.	191
B.1	The PMF used for the 2D-BD simulations.	192
B.2	Discrete probability distribution of membrane crossings, based on 593 independent 2-dimensional Brownian dynamics stochastic simulations, each $50 \mu\text{s}$ in duration.	192

B.3	Assessment of the automatic optimization of initial positions for short unbiased 2-dimensional Brownian dynamics stochastic simulations.	193
B.4	Cumulative distribution function (CDF) of the initial positions obtained by the automatic optimization scheme for short unbiased 2D-Brownian dynamics stochastic simulations.	193
B.5	Unwrapped committor probability for each sucrose and membrane system, which were extended to cover one additional periodic image in the positive and negative directions along z	194
B.6	Histogram of the initial z positions of each short unbiased trajectory used for re-weighting the time-correlation functions for each set of sucrose MD simulations.	195
B.7	Relative error of the committor time-correlation function within each z_n bin for each force field and membrane system of the sucrose MD simulations.	195
B.8	Illustration of error analysis and optimization of the C36 DLPC system of sucrose permeation.	196
B.9	Illustration of error analysis and optimization of the Drude DLPC system of sucrose permeation.	196
B.10	Integral of the position autocorrelation function for each membrane and force field system for several umbrella windows.	197
B.11	Hydration number calculated from the trajectory of each window of the US simulations of sucrose in DOPC and DLPC membranes, with the C36 and Drude force fields.	198
C.1	Comparison of simple and modified loss functions of the MLP in each umbrella window for water across a DLPC membrane.	200
C.2	Position-dependent diffusivity ($D(z)$) and PMF results of equilibrium simulations of the numerical GLE simulations with EM-3 and EM-3m integrator using the position-dependent memory function.	201
C.3	Structure of the MLP developed by Russo et al. ¹³⁴ for optimizing memory functions, with $N = 2$ neurons in the hidden layer.	202
C.4	Velocity autocorrelation function for each replicate and umbrella window from the MD simulations.	203
C.5	Force-velocity ($C_{Fv}(t)$) time-correlation function for each replicate and umbrella window from the MD simulations.	204
C.6	Optimized memory functions for each replicate and umbrella window from the MD simulations.	205
C.7	Replicate 1: Laplace transform of the Woolf-Roux diffusivity from the MD simulations.	206
C.8	Replicate 2: Laplace transform of the Woolf-Roux diffusivity from the MD simulations.	207
C.9	Replicate 3: Laplace transform of the Woolf-Roux diffusivity from the MD simulations.	208
C.10	The integral of the position-autocorrelation function for each window of the umbrella sampling MD simulations.	209

C.11	Replicate 1: Laplace transform of the Woolf-Roux diffusivity from the GLE simulations.	210
C.12	Replicate 2: Laplace transform of the Woolf-Roux diffusivity from the GLE simulations.	211
C.13	Replicate 3: Laplace transform of the Woolf-Roux diffusivity from the GLE simulations.	212
C.14	The integral of the position-autocorrelation function for each window of the GLE umbrella sampling simulations.	213
C.15	Velocity autocorrelation function for each replicate and umbrella window of the GLE simulations.	214
C.16	Force-velocity ($C_{Fv}(t)$) time-correlation function for each replicate and umbrella window of the GLE simulations.	215
C.17	Results of the Grote-Hynes analysis, using an inverted parabolic fit to the PMF from MD simulations of water permeation, and the memory functions extracted from the center $z = 0$ window of the umbrella sampling MD simulations.	217
D.1	CZAR gradient ²⁰³ output from the eABF algorithm in colvars in NAMD 2.14 ^{194;195} for each ionization state of nicotine and varenicline.	221
D.2	Histogram of eABF sampling used for computing the CZAR gradients, shown for each ionization state of nicotine and varenicline.	222
D.3	Unsymmetrized PMFs for each ionization state of nicotine and varenicline.	223
D.4	Mean-square deviation (MSD) diffusivity for all ionization states of nicotine and varenicline before symmetrization.	224
D.5	Time-fractional position dependent diffusivity for lag times of 5 and 10 ps, along with the MSD diffusivity for a lag time of 20 ps for the three ionization states of nicotine.	225
D.6	Snapshots from the converged eABF simulations for each ionization state of nicotine, illustrating the extent to which water enters the membrane in each instance.	226
D.7	Hydration numbers for each ionization state of nicotine along the z -axis.	227
D.8	Boltzmann-weighted average diffusivity profiles over a range of pH values for nicotine and varenicline.	228
D.9	Empirical assessment of the relationship between barrier height, pK_a , and the ratio of pH-partition hypothesis weighted permeability of a neutral and protonated state.	230

LIST OF TABLES

2.1	Summary of BD and BD _e simulations	32
2.2	Calculated permeability for inverted harmonic barrier with exponential memory	38
2.3	Permeability from BD simulations with memory effects (cm/s)	42
3.1	Summary of 2D-Brownian dynamics simulations	63
3.2	Sucrose permeability from MD simulations for DOPC and DLPC membranes, in units of $\times 10^{-6}$ cm/s	76
3.3	Experimental sucrose permeability	79
4.1	Equilibrium GLE simulations	93
4.2	Permeability of DLPC to water, units of $\times 10^{-3}$ cm/s	111
6.1	Mean reactive transit times, $\langle \tau_r \rangle$, ns	152
6.2	Specific POPC membrane permeability to each ionization state of nicotine and varenicline at 308 K, cm/s	158
C.1	Grote-Hynes permeability from the Woolf-Roux, Hummer, and MLP Laplace space memory functions	218

ACKNOWLEDGMENTS

I would like to acknowledge the people who made the completion of this dissertation possible: my adviser Benoît Roux, my defense committee Aaron Dinner and Andrei Tokmakoff, my collaborators Christophe Chipot and Richard Pastor, and the many members of the Roux, Voth, and Dinner groups who have helped me along the way. I would also like to specially thank my undergraduate adviser John Straub and mentor George Pantelopulos, who I credit with setting me upon the path towards the completion of my PhD.

ABSTRACT

A rigorous theory for the treatment of biological membrane permeation by small molecules is developed. The inhomogeneous solubility-diffusion model that is commonly used to compute membrane permeability from molecular dynamics simulations is lacking because it neglects the effects of memory on the rate of barrier crossing. Memory effects are accounted for through the extension of transition path theory to membrane permeability through time-correlation functions of the committor probability. Special attention is paid to the permeation of water because theoretical predictions can be validated based on highly accurate brute force simulations. Using a deep learning framework, the position-dependent memory function of water was extracted from umbrella sampling molecular dynamics simulations. The inhomogeneous solubility-diffusion model, Grote-Hynes theory, and direct simulation of the generalized Langevin equation with position-dependent memory are invoked to demonstrate that memory effects are most likely negligible for water permeation. An additional consideration is the permeation of nicotine, which is a small ionizable molecule. The pH-partition hypothesis is confirmed for nicotine based on a transition path theory treatment of the permeation pathway that incorporates the experimental rates of protonation. In sum, several permeation processes are subjected to rigorous theories of statistical mechanics and transition path theory in order to confirm the validity of classic assumptions.

CHAPTER 1

INTRODUCTION

1.1 Biological membrane permeability

The movement of molecules across a membrane is described by the constitutive relationship,¹

$$J = P\Delta c \tag{1.1}$$

where J is the flux across the membrane from side A to side B, Δc is the difference between the concentration on each side, and P is the proportionality constant known as the permeability. Because the flux has units of quantity per time through an area, and concentration has units of quantity over volume, the permeability is left with units of length per time. The homogeneous solubility-diffusion model,¹

$$P = KD/L \tag{1.2}$$

defines the permeability in terms of the partition coefficient, K , and diffusion constant, D , for a membrane of thickness L . These properties depend on the membrane and the molecule that is crossing it, known as the permeant. The partition coefficient is the ratio of the solubility of the permeant in the membrane to that of the permeant in water. Permeability is therefore a property of a membrane and permeant pair that is related to both thermodynamic driving forces and transport kinetics. The proportionality between K and P , where K is the partition coefficient for a given molecule between water and hydrocarbon, is known as Overton's Rule, which was established around 1900.^{1;2}

In the context of biology, membranes are composed of lipids that are arranged in symmetric two-layered structures.³ Across these so-called lipid bilayers, the properties of the effective medium around a given permeant vary greatly: at the very edge of the membrane

system, the medium is purely water; the water region touches the polar heads of the lipid molecules; the polar heads are bonded to the lipid tails; at the very center there is a space where tails of the upper and lower leaflets interact.⁴ These regions tend to overlap with each other as the membrane fluctuates, resulting in a smooth gradient of media across the membrane on average, and a similarly smooth position-dependent diffusion coefficient.⁵ It can also be expected that the solubility of the permeant will vary smoothly across the overlapping slices of media, from the water and polar heads, to the hydrophobic interior.⁶

It is desirable to be able to incorporate the position-dependence of the diffusivity and solubility of the permeant across the membrane into the model of permeation. Such an expression can be derived from Smoluchowski diffusion equation across the membrane,⁷

$$\frac{\partial P(z, t)}{\partial t} = -\frac{\partial}{\partial z} \left(-\frac{D(z)}{k_B T} \frac{\partial W(z)}{\partial z} P(z, t) - D(z) \frac{\partial P(z, t)}{\partial z} \right) \quad (1.3)$$

where $P(z, t)$ is the time-dependent 1-dimensional probability distribution of the permeant across the membrane, $D(z)$ is the position-dependent diffusivity, and $W(z)$ is the potential of mean force (PMF). The closed-form expression of the permeability, first discovered by Diamond and Katz in 1974, is known as the inhomogeneous solubility-diffusion (ISD) model,⁸

$$P = \left[\int_{-L/2}^{L/2} dz \frac{e^{[W(z)-W(-L/2)]/k_B T}}{D(z)} \right]^{-1} \quad (1.4)$$

where the membrane is centered at $z = 0$. In eq 1.3 and eq 1.4, the permeation process was tacitly projected onto a reaction coordinate z . In a common convention, the plane of the membrane is oriented to be perpendicular to the Cartesian z -coordinate. The reaction coordinate z is then represented by a collective variable, which is generally the Cartesian z component of the distance between the center of mass of the permeant and the center of mass of the membrane.

The PMF can be defined in terms of the marginal probability distribution of z . Starting

with the potential energy function $U(z, \mathbf{Y})$, where $\mathbf{Y}$ is all other degrees of freedom, the configurational probability distribution is,⁹

$$P(z, \mathbf{Y}) = \frac{e^{-U(z, \mathbf{Y})/k_{\text{B}}T}}{\int dz \int d\mathbf{Y}' e^{-U(z', \mathbf{Y}')/k_{\text{B}}T}} \quad (1.5)$$

The marginal probability is then,⁹

$$p(z) = \int d\mathbf{Y}' P(z, \mathbf{Y}') \quad (1.6)$$

which is the integral of the configurational probability distribution over all degrees of freedom besides z . In practice, the marginal probability distribution is essentially a histogram of sampling in bins along z , or, $\langle \delta(z - z') \rangle$. The distribution $p(z)$ is also the (stationary) solution of eq 1.3 for which $\partial P(z, t)/\partial t = 0$. The PMF itself is,

$$W(z) = -k_{\text{B}}T \ln[p(z)] \quad (1.7)$$

The PMF can be computed from molecular dynamics simulations, usually involving enhanced sampling approaches.¹⁰ The first application of MD simulations to estimate biological membrane permeability with eq 1.4 was completed by Marrink and Berendsen in 1994.⁶

1.2 Statistical mechanics and transition path theory

1.2.1 Brownian dynamics and the ISD model

Before beginning the discussion of the application of transition path theory to membrane permeability, it is important to emphasize the assumptions of the ISD model and the conditions under which it can be applied. In order to build intuition, a Brownian dynamics

simulation is used as an illustration. The motion of a Brownian permeant is governed by,⁹

$$z(t + \Delta t) = z(t) - \frac{D(z)}{k_B T} \left(\frac{\partial W(z)}{\partial z} \right) \Delta t + \left(\frac{\partial D(z)}{\partial z} \right) \Delta t + \sqrt{2D(z)\Delta t} \mathcal{N}_t \quad (1.8)$$

where Δt is the timestep, $D(z)$ is the position-dependent diffusivity, $W(z)$ is the PMF, and $\mathcal{N}_t$ is a Gaussian random number. The derivative of the position-dependent diffusivity, $\partial D(z)/\partial z$, corresponds to the force-like term which corrects the spurious drift.

Consider a Brownian dynamics simulation which has been parameterized with the PMF and position-dependent diffusivity extracted from MD simulations of a permeant across a membrane. Due to computational simplicity of Brownian dynamics, is possible to numerically integrate long, equilibrium simulations by brute force. That is, it is feasible to propagate eq 1.8 for millions of steps, using a timestep of about 1 fs. To construct the simplified membrane, the z coordinate can be constructed with half harmonic restraints at each edge of the system, with the PMF centered at $z = 0$. It is also necessary to define the edges of the membrane at $\pm L/2$, which can be placed where the PMF reaches zero on either side. For this system, a membrane crossing occurs each time the Brownian permeant begins at $z < -L/2$ or $z > L/2$, and ends at $z > L/2$ or $z < -L/2$, respectively. For a given equilibrium simulation of length $\mathcal{T}$, there will be a number of crossings, $N_c(\mathcal{T})$ corresponding to a Poisson distribution. The simulation has converged when there have been approximately 100 crossings, which corresponds to a standard error of around 10%.^{11;12} The permeability can be estimated directly from the number of crossings,

$$P = \frac{N_c(\mathcal{T})}{2\mathcal{T}\pi(-L/2)} \quad (1.9)$$

where $\pi(-L/2)$ is the 1-dimensional probability of the permeant at the edge of the membrane,

$$\pi(-L/2) = \left[\int_{z_{min}}^{z_{max}} dz e^{-W(z)/k_B T} \right]^{-1} \quad (1.10)$$

where z_{min} and z_{max} are the locations of the lower and upper half harmonic boundaries of the system, respectively. The counting permeability based on the Brownian dynamics simulation will converge to the ISD permeability of eq 1.4 in the limit of many crossing events.

1.2.2 *The generalized Langevin equation*

In some situations, memory effects associated with the reaction coordinate z may lead to inconsistencies between the ISD and the counting permeability. An example of a deviation between the rate of barrier crossing and that assumed by the ISD model can also be modeled with numerical simulations. The example involves introducing memory into the Brownian dynamics simulations through an overdamped generalized Langevin equation (GLE).¹³ The generalized Langevin equation is given by,¹⁴

$$m\ddot{z}(t) = -\frac{\partial W}{\partial z} - \int_0^t dt' M(t-t') \dot{z}(t') + f(t) \quad (1.11)$$

where m is the mass of the permeant, $W(z)$ is the potential of mean force (PMF), $M(t)$ is the memory function, and $f(t)$ is a random noise obeying the second fluctuation-dissipation theorem, $\langle f(0)f(t) \rangle = k_B T M(t)$. The diffusivity is related to the memory function via the Einstein relation,⁹

$$D = \frac{k_B T}{\int_0^\infty M(t) dt} \quad (1.12)$$

If the memory function is chosen to be a delta function, $M(t) = \delta(t)k_{\text{B}}T/D$, and the overdamped limit $m \rightarrow 0$ is taken, Brownian dynamics is recovered,

$$\begin{aligned} 0 &= -\frac{\partial W}{\partial z} - \int_0^t dt' \delta(t-t') \frac{k_{\text{B}}T}{D} \dot{z}(t') + f(t) \\ \frac{k_{\text{B}}T}{D} \dot{z}(t) &= -\frac{\partial W}{\partial z} + f(t) \\ \dot{z}(t) &= -\frac{D}{k_{\text{B}}T} \frac{\partial W}{\partial z} + g(t) \end{aligned} \quad (1.13)$$

where $g(t)$ is a Gaussian random noise. It is also possible to obtain overdamped dynamics while retaining the memory function. A mathematically and computationally convenient choice for the memory function is a positive, decaying exponential,

$$M(t) = \gamma_z \delta(t) + K_e e^{-t/\tau_e} \quad (1.14)$$

where γ_z is the Langevin-like friction term, K_e is the memory amplitude, $K_e \tau_e = \gamma_e$, and γ_e is the friction due to the exponential term. The effective diffusivity is then,

$$\begin{aligned} D &= \frac{k_{\text{B}}T}{\int_0^\infty [\delta(t) \gamma_z + K_e e^{-t/\tau_e}] dt} \\ &= \frac{k_{\text{B}}T}{\gamma_z + \gamma_e} \end{aligned} \quad (1.15)$$

Inserting the exponential memory function into the overdamped GLE yields,

$$\dot{z}(t) = -\frac{1}{\gamma_z} \frac{\partial W}{\partial z} - \frac{1}{\gamma_z} \int_0^t dt' K_e e^{-(t-t')/\tau_e} \dot{z}(t') + g(t) \quad (1.16)$$

In this form, the GLE is difficult to integrate numerically due to the convolution integral, which depends on the entire history of velocities, and becomes more expensive to calculate as the simulation progresses. Through a method known as Markovian embedding,¹⁵ it is

possible to compute the “running sum” of the convolution integral with an extended variable for a memory function that is a positive, decaying exponential. Consider the extended variable,

$$z(t) - z_e(t) = \int_0^t dt' e^{-(t-t')/\tau_e} \dot{z}(t') \quad (1.17)$$

The time derivative of the the above expression results in,

$$\begin{aligned} \frac{\partial}{\partial t}(z(t) - z_e(t)) &= \frac{\partial}{\partial t} \int_0^t dt' e^{-(t-t')/\tau_e} \dot{z}(t') \\ \dot{z}(t) - \dot{z}_e(t) &= \dot{z}(t) + \int_0^t dt' \frac{\partial}{\partial t} e^{-(t-t')/\tau_e} \dot{z}(t') \\ &= \dot{z}(t) - \frac{1}{\tau_e} \int_0^t dt' e^{-(t-t')/\tau_e} \dot{z}(t') \\ &= \dot{z}(t) - \frac{1}{\tau_e} (z(t) - z_e(t)) \\ \dot{z}_e(t) &= \frac{1}{\tau_e} (z(t) - z_e(t)) \end{aligned} \quad (1.18)$$

which uses the Leibniz integral rule. After inserting eq 1.18 back into eq 1.16, the equations of motion for the overdamped GLE with exponential memory become,

$$z(t + \Delta t) = z(t) - \frac{1}{\gamma_z} \frac{\partial W}{\partial z} \Delta t - \frac{1}{\tau_z} (z(t) - z_e(t)) \Delta t + \sqrt{2D_z \Delta t} \mathcal{N}_t \quad (1.19)$$

$$z_e(t + \Delta t) = z_e(t) + \frac{1}{\tau_e} (z(t) - z_e(t)) \Delta t + \sqrt{2D_e \Delta t} \mathcal{N}_t \quad (1.20)$$

where $\tau_z = \frac{k_B T}{D_z K_e}$, $D_z = k_B T / \gamma_z$, and N_t is a normally distributed random number. The random noise terms are inserted to ensure that each equation of motion satisfies the fluctuation-dissipation theorem. In this case, z is the main reaction coordinate because it contains the mean force term. The other coordinate, z_e contributes memory effects and ultimately affects the rate of barrier crossing.

It is worth noting that the set of overdamped equations that were obtained with Markovian embedding are equivalent to 2-dimensional Brownian dynamics equations that are cou-

pled together in a harmonic potential.¹³ For the 2-dimensional Brownian dynamics representation, the coordinates z and z_e are coupled by the 2-dimensional potential energy surface given by,

$$U(z, z_e) = W(z) + \frac{1}{2} K_e (z - z_e)^2 \quad (1.21)$$

and the force on each coordinate is,

$$\begin{aligned} F_z &= -\frac{\partial}{\partial z} U(z, z_e) \\ &= -\frac{\partial W}{\partial z} - K_e (z - z_e) \end{aligned} \quad (1.22)$$

$$F_{z_e} = -\frac{\partial}{\partial z_e} U(z, z_e) \quad (1.23)$$

$$= K_e (z - z_e) \quad (1.24)$$

which correspond to the forces in eq 1.19 and eq 1.20. The effective exponential memory function with respect to z can be shown by fixing the particle at $z = 0$ and computing the force-force time-correlation function. When z is fixed at zero, the force-force time-correlation function is given by,

$$\langle F_z(t) F_z(0) \rangle = \langle K_e z_e(t) K_e z_e(0) \rangle \quad (1.25)$$

where the position autocorrelation function for z_e when z is fixed at zero is,

$$\frac{\partial}{\partial t} \langle z_e(t) z_e(0) \rangle = -\frac{1}{\tau_e} \langle z_e(t) z_e(0) \rangle \quad (1.26)$$

$$\langle z_e(t) z_e(0) \rangle = \langle z_e^2 \rangle e^{-t/\tau_e} \quad (1.27)$$

This above expression was obtained by taking the time derivative and then multiplying both sides of eq 1.20 by $z_e(0)$. In a harmonic potential, $\langle z_e^2 \rangle = k_B T / K_e$. The friction on

z due to the coupling with z_e is related to both the memory function and the force-force autocorrelation function. Their equivalence can be shown by,⁹

$$\gamma_e = \int_0^\infty dt M(t) \quad (1.28)$$

$$= \frac{1}{k_B T} \int_0^\infty dt \langle F_z(t) F_z(0) \rangle \quad (1.29)$$

$$= \frac{1}{k_B T} \int_0^\infty dt \langle K_e z_e(t) K_e z_e(0) \rangle \quad (1.30)$$

$$= \int_0^\infty dt K_e e^{-t/\tau_e} \quad (1.31)$$

$$= \frac{k_B T}{D_e} \quad (1.32)$$

which corresponds to the expected memory function and friction due to the z_e coordinate.

1.2.3 *The effect of memory on the rate of barrier crossing*

In the previous section, a 2-dimensional Brownian dynamics framework was developed for incorporating memory effects into overdamped dynamics. If long, equilibrium simulations are conducted by numerical integration of eq 1.19 and eq 1.20, the counting permeability based on the number of crossings events observed along z is expected to deviate from the ISD permeability, in contrast to what is expected from pure Brownian dynamics. For the ISD model, the difficulty comes from estimating the effective diffusivity of the particle as it crosses the barrier. The effective diffusivity of eq 1.15 serves as a lower limit. Grote-Hynes theory clearly illustrates the mechanism by which the memory function can cause the ISD model to underestimate the true rate of barrier crossing. The approach of Grote-Hynes theory is able to incorporate both the timescale of crossing the barrier and the decay of the memory function to the overall rate.¹⁶ Due to the overdamped dynamics and exponential memory function, it is possible to analytically determine the exact permeability of the 2-dimensional Brownian dynamics simulations with Grote-Hynes theory. The analytical permeability will

precisely match the counting permeability if the additional criteria of a strictly inverted parabolic PMF is imposed. This 2-dimensional Brownian dynamics framework is further explored in Chapter 2 via Grote-Hynes theory and numerical simulations.

In practice, all-atom MD simulations provide a more complex picture of memory effects and permeation. In the general case of water permeation, the counting permeability can be estimated from spontaneous crossing events in long, equilibrium simulations. In Chapter 2, it is shown how the tools of transition path theory¹⁷ can be used to compute the exact permeability based on the committor framework.¹⁸ In Chapter 3, the committor framework is extended for enhanced sampling simulations, which employs a combination of umbrella sampling and short unbiased trajectories. In Chapter 4, memory functions are extracted directly from umbrella sampling MD simulations, which allows for simulating the full GLE to assess the effect of memory on the permeability. In Chapter 5, an enhanced sampling approach combining the full GLE, transition path theory, and short unbiased trajectories is developed.

1.3 Comparing simulations with experiment

In recent years, the theory of biological membrane permeability has been developed primarily for the application of drug design.^{19;20} In common practice, a relevant measure of the permeability of drugs taken orally is assumed to be that of Caco-2 cells,²¹ which model human intestinal lining. Alternative experimental methods, such as vesicle fluorescence²² and pH-dependent probes,²³ provide single-component membranes and vesicles of relatively small volumes. In general, the assumptions of vesicle experiments make them more readily comparable with the permeability results of MD simulations. Whether the membrane is pristine and composed of only a single type of lipid, or it is a biologically functional *fluid mosaic*,²⁴ it is important for the permeation modeler to be aware of experimental assumptions when assessing the accuracy of simulation results.

It is instructive to derive an expression for the permeability based on a typical vesicle experimental setup. Beginning with the constitutive relationship of eq 1.1, consider the flux of the permeant into the vesicle, $J_{\text{out} \rightarrow \text{in}}$, based on the concentration outside c_{out} , and inside, c_{in} , and the permeability P . Assume the vesicle is a large unilamellar vesicle (LUV), with radius r_L , surface area $A_L = 4\pi r_L^2$, and volume $V_L = 4/3\pi r_L^3$. If the empty vesicle is suddenly exposed to an ideal solution with a concentration of c_{out} of the permeant at $t = 0$, the time-dependent concentration of the permeant inside and outside of the vesicle can be modeled with,²⁵

$$\frac{dN_{\text{in}}(t)}{dt} = -\frac{dN_{\text{out}}(t)}{dt} = J_{\text{out} \rightarrow \text{in}} A_L \quad (1.33)$$

Assuming that the volume of the solution is much bigger than the volume of the LUV, then $c_{\text{out}}(t) \approx c_{\text{out}}$, and

$$c_{\text{in}}(t) = c_{\text{out}}(1 - e^{-3Pt/r_L}) \quad (1.34)$$

where the relaxation time is $\tau = r_L/3P$. For an LUV, $r_L \approx 50$ nm. It is necessary for the experimentalist to track the concentration of the permeant inside of the LUV over time, which is possible with various methods.²²

The interplay between permeability, vesicle volume, and experimental observations must be treated carefully. For instance, in the case of weak acids and bases, it is possible to estimate the permeability based on the change in fluorescence of vesicles with pH-sensitive dyes inside.²³ However, it was shown by Sezer and Oruç²⁵ that for LUVs of the standard radius, the timescale for c_{in} to equal c_{out} can be shorter than the timescale of changing the pH inside the vesicle. It is reasonable that protonation kinetics are rate limiting in the permeation process of a weak acid or base, in its strictly neutral form.²⁶ This observation casts doubt on the applicability of using constant pH molecular dynamics to model the membrane permeation of small molecules,^{27;28} as well as the utility of vesicle fluorescence

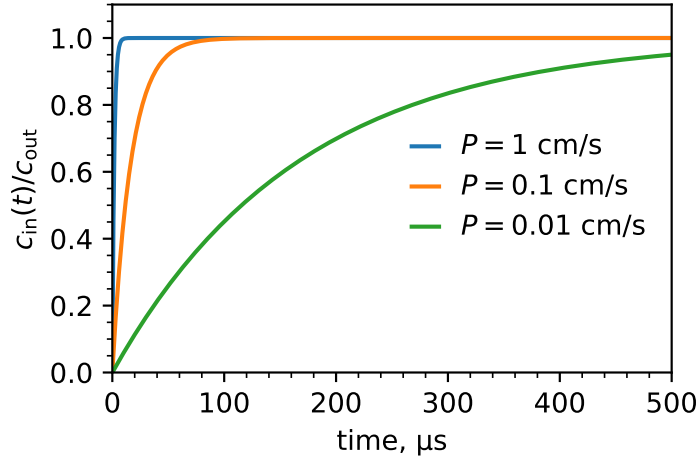


Figure 1.1: The normalized time-dependent concentration of molecules with varying permeability coefficients inside a hypothetical LUV with a radius of 50 nm.

with pH-dependent probes.²³

Another special case for experimental measurements of permeability concerns that of water itself. For water, it is possible to estimate the permeability by exposing vesicles to an osmotic gradient by adding an “impermeable” chemical species to the solution, such as sucrose.²⁹ The permeability is then derived from the rate of change of the volume of the vesicle, which shrinks due to outward flux of water caused by the osmotic pressure. The change in volume of the vesicle can be measured by the change in fluorescent signal of self-quenching entrapped carboxyfluorescein.²⁹ For this setup, the kinetic model of eq 1.34 must be modified. We assume that the chemical species used to impose the osmotic pressure never enters the vesicle, therefore $c_{in} = 0$. The volume of the bulk solution is still much larger than the volume of the vesicles, so the $c_{out}(t) \approx c_{out}$ assumption remains. The flux of water out of the vesicle is simply $J_{in \rightarrow out} = P c_{out}$. The flux can be expressed in the quantity of water molecules passing through the surface area of the vesicle over time,

$$\frac{dN_w(t)}{dt} = -A_L(t) P c_{out} \quad (1.35)$$

where $N_w(t)$ is the number of water molecules inside the vesicle at time t . Then both sides are divided by the concentration of water, c_w ,

$$\frac{dV(t)}{dt} = -\frac{1}{c_w}A_L(t)Pc_{\text{out}} \quad (1.36)$$

where, $V(t) = N_w(t)/c_w$.

In general, comparing the permeability coefficients estimated with MD simulations with LUV experiments is straightforward. For water permeation, the agreement between simulations and experiment can be used as a benchmark for force field accuracy³⁰ because the assumptions involved are so strong. Water permeability can be estimated from equilibrium simulations because a statistically meaningful number of crossings can occur on a reasonable timescale due to the high concentration of water in the simulation. In LUV experiments, water permeability has been determined for many different single component membranes, allowing for very direct comparisons in many cases.^{29;31} Weak acid and base permeation can lead to additional complications compared to water due to protonation kinetics.²⁵ A thorough discussion of the permeability of the weak base nicotine, the impact of protonation kinetics, and comparisons with relevant experiments is provided in Chapter 6. A focused analysis on the permeability of the singly protonated (dominant at neutral pH) species of nicotine is given in Chapter 7.

CHAPTER 2

STATISTICAL MECHANICAL THEORIES OF MEMBRANE PERMEABILITY

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<https://pubs.acs.org/doi/full/10.1021/acs.jpcb.4c05020>

ABSTRACT – A popular theoretical framework to compute the permeability coefficient of a molecule is provided by the classic Smoluchowski-Kramers treatment of the steady-state diffusive flux across a free energy barrier. Within this framework, commonly referred to as “inhomogeneous solubility-diffusion” (ISD), the permeability, P , is expressed in closed form in terms of the potential of mean force and position-dependent diffusivity of the molecule of interest along the membrane normal. In principle, both quantities can be calculated from all-atom MD simulations. Although several methods exist for calculating the position-dependent diffusivity, each of these is at best an estimate. In addition, the ISD model does not account for memory effects along the chosen reaction coordinate. For these reasons, it is important to seek alternative theoretical formulations to determine the permeability coefficient that are able to account for the factors ignored by the ISD approximation. Using Green-Kubo linear response theory, we establish the familiar constitutive relation between the flux density across the membrane and the difference in the concentration of a permeant molecule, $j = P \Delta C$. On this basis, we derive a time-correlation function expression for the non-equilibrium flux across a membrane that is reminiscent of the transmission coefficient in the reactive flux formalism treatment of transition rates. An analysis based on the transition path theory framework is exploited to derive alternative expressions for the permeability

coefficient. The different strategies are illustrated with stochastic simulations based on the generalized Langevin equation, in addition to unbiased molecular dynamics simulations of water permeation of a lipid bilayer.

2.1 Introduction

Calculating membrane permeability from first principles is an engaging problem of biophysical research from both theoretical and practical perspectives.^{19;32} It is also of great interest to pharmacokinetics and drug development.^{20;19;33} A popular theoretical framework to compute the permeability coefficient of a molecule is provided by the classic Smoluchowski-Kramers treatment of the steady-state diffusive flux across a free-energy barrier.^{7;34} Within this framework, the permeability, P , is expressed as,

$$\frac{1}{P} = \int_{-L/2}^{L/2} dz \frac{e^{+[W(z)-W(-L/2)]/k_B T}}{D(z)} \quad (2.1)$$

where L is the membrane thickness, and $D(z)$ and $W(z)$ are the position-dependent diffusivity and the potential of mean force (PMF) of the molecule of interest along the membrane normal, z , respectively. The membrane thickness, L , in eq 2.1 should be chosen such that the PMF, $W(z)$, is equal to a constant when $|z| > L/2$ in the bulk region (the constant can be set to zero for convenience). This theoretical framework, commonly referred to as “inhomogeneous solubility-diffusion” (ISD) since the pioneering work of Diamond and Katz,⁸ has been applied to a variety of molecules.^{6;35;36;37;38;39;40} In eq 2.1, both the PMF, $W(z)$, and diffusivity, $D(z)$, can be calculated from MD simulations. The PMF is a concept that is rigorously defined on the basis of equilibrium statistical mechanics,⁴¹ and a host of enhanced sampling methods can be used for its determination.^{42;43;10;44;45;46} The diffusivity, on the other hand, is a dynamical transport coefficient, whose validity can clearly be context-dependent. When the dynamics of a molecule is truly diffusive on appropriate length scales

and timescales, $D(z)$ can be determined from the evolution of the mean squared deviation (MSD) of displacements over a short lag time. However, the significance of an estimate based on the MSD becomes questionable if the displacement of the molecule over one lag time is incompatible with the variations of the free energy landscape. An important aspect of ISD is that it is based on the assumption that the dynamics of the permeant molecule along the z -axis can be represented by a memory-less diffusive process. Generally, the diffusivity D is estimated from the evolution of the MSD as a function of time, $\langle \Delta z^2 \rangle \sim 2Dt$. However, observing this straightforward limiting behavior in MD simulations is often problematic. Dynamics may appear slower than any classical diffusive process over a range of timescales, suggesting the existence of subdiffusive processes,⁴⁷ characterized by $\langle \Delta z^2 \rangle \sim K_\alpha t^\alpha$, where K_α is a generalized diffusion coefficient of fractional order α , such that $0 \leq \alpha \leq 1$. Experimental work suggests that subdiffusion can be relevant on timescales reaching many seconds in multicomponent membranes.⁴⁸ These issues are perhaps the most critical limitations of the ISD model.⁴⁹

In this context, it is important to consider alternative strategies to determine the permeability of molecules that go beyond the assumptions of the ISD theoretical framework. Perhaps the most direct and straightforward treatment of permeation consists of counting spontaneous membrane crossing events,^{50;12;1;51;52} although this strategy is best suited for small molecules with high intrinsic membrane permeability. A more systematic strategy to collect information about unbiased membrane crossings is to rely on the transition path sampling (TPS) framework,⁵³ although the approach is limited to small molecules as it becomes computationally intractable for slowly permeating molecules. Markov state models (MSM),^{54;55} Milestoning,^{56;57;58;59;60} and weighted ensemble⁶¹ represent three additional alternative approaches. To some extent, these approaches picture the dynamics of the permeant molecule in terms of a stochastic process along the z -axis that can be characterized from all-atom MD simulations using enhanced sampling strategies.

In the present work, we first revisit the problem of permeation, starting from Green-Kubo linear response theory^{62;63;64;65} to derive time-correlation function expressions for the non-equilibrium flux of a permeant molecule's diffusing across a membrane. The analysis recovers the familiar constitutive relation, $j = P \Delta C$, relating the flux density j to the difference in the concentration, ΔC , of the permeant molecule across the membrane. The expression for the permeability coefficient can be cast in a form that is reminiscent of the reactive flux formalism,⁶⁶ with a transmission coefficient κ . Pursuing this line of thought, it is shown that memory effects can be understood by following a treatment similar to the Grote-Hynes theory of transition rates.¹⁶ An analysis based on the transition path theory (TPT) framework^{67;17;68;69;70;18} is then exploited to derive alternative expressions for the permeation coefficient. The TPT formulation based on the committor time-correlation function is illustrated with stochastic simulations based on the generalized Langevin equation (GLE),^{71;14;72} and long unbiased MD simulations of water permeation.

2.2 Theoretical Developments

2.2.1 Membrane permeability and Green-Kubo linear response

We consider a system with two volumes, a and b , separated by a membrane governed by the reference Hamiltonian $\mathcal{H}_0$. Averages based on the reference Hamiltonian $\mathcal{H}_0$ are represented as, $\langle \dots \rangle_0$. We assume that a permeant molecule is present at a low concentration in the system. At equilibrium, the concentration of the permeant molecule in the bulk solution is the same on both sides of the membrane, and its mean one-dimensional density is $\langle \rho(z_a) \rangle_0 = \langle \rho(z_b) \rangle_0 = \rho_0$.

We introduce the population operators N_a and N_b equal to the total number of permeant molecules in the regions a and b , and zero otherwise, respectively. For example, in the case

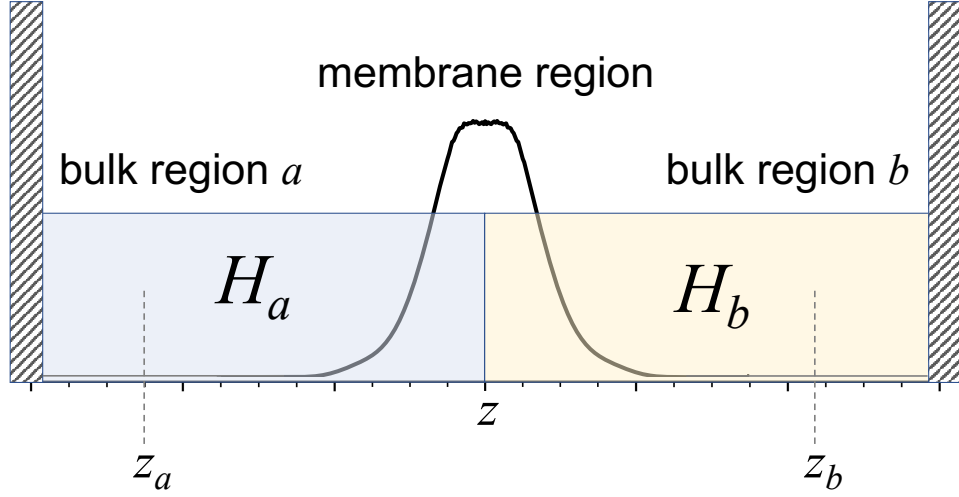


Figure 2.1: Physical situation of a membrane separating a bulk region a (blue) and a bulk region b (yellow) containing a permeant molecule. The indicator function H_a (H_b) is equal to 1 if the permeant molecule i is on side a (b), and equal to zero otherwise. By construction, all space is covered by the two indicator functions, with $H_a + H_b = 1$. The concentration of permeant molecule at the positions z_a and z_b in the bulk region away from the membrane serves as a reference to establish the constitutive relation $j = P\Delta C$ of membrane permeation.

of the population operator for region a ,

$$N_a(t) = \sum_i^{N_{\text{tot}}} H_a(\mathbf{r}_i(t)) \quad (2.2)$$

where the indicator functions $H_a(\mathbf{r}_i)$ are equal to 1 if the permeant molecule i is in region a , and equal to zero otherwise, and

$$N_b(t) = \sum_i^{N_{\text{tot}}} H_b(\mathbf{r}_i(t)) \quad (2.3)$$

where the indicator functions $H_b(\mathbf{r}_i)$ are equal to 1 if the permeant molecule i is in region b , and equal to zero otherwise. All space is covered by construction with $H_a + H_b = 1$, and $N_a + N_b = N_{\text{tot}}$. To create a small imbalance in the concentration of permeant molecules between the two sides of the membrane, we assume that the system is initially prepared with

the Hamiltonian $\mathcal{H}_0 - \epsilon N_a$, where ϵ is a small positive energy constant. The perturbation increases the concentration of permeant molecules on the side a relative to the side b . In the perturbed system, the mean one-dimensional density of permeant molecules at point z' is $\langle \rho(z') \rangle = \sum_i \langle \delta(z' - z_i) \rangle$. Suddenly, at $t = 0$, the perturbation is switched off and the dynamical evolution from then on is governed by the Hamiltonian $\mathcal{H}_0$ to relax back to a uniform concentration of the permeant molecule on both sides. We monitor the evolution of the population operator $N_b(t)$ of the permeant molecules as a function of time for $t > 0$. If $\epsilon > 0$, the net flux J from a to b caused by suddenly turning off the perturbation $-\epsilon N_a$ is related to the rate of change of the population on side b , $\langle \delta \dot{N}_b(t) \rangle$,

$$\langle \delta \dot{N}_b(t) \rangle = \frac{\epsilon}{k_B T} \langle \delta \dot{N}_b(t) \delta N_a(0) \rangle_0 \quad (2.4)$$

where $\delta N_a = N_a - \langle N_a \rangle_0$, and $\delta N_b = N_b - \langle N_b \rangle_0$. This is the net number of permeant molecules crossing the membrane from region a to region b per unit of time due to the perturbation $-\epsilon N_a$ ($\delta \dot{N}_b$ and δN_a can be swapped in this expression by time symmetry). We can rewrite eq 2.4 by relating ϵ to the ratio of one-dimensional density for $t < 0$, $\langle \rho(z_a) \rangle$ and $\langle \rho(z_b) \rangle$,

$$\begin{aligned} \frac{\langle \rho(z_a) \rangle}{\langle \rho(z_b) \rangle} &= e^{\epsilon/k_B T} \\ \frac{\left(\langle \rho(z_a) \rangle - \langle \rho(z_b) \rangle \right)}{\rho_0} &\approx \frac{\epsilon}{k_B T} \end{aligned} \quad (2.5)$$

Therefore, the net long-time stationary flux after initial transient effects have receded is,

$$\begin{aligned}
J &= \lim_{t \rightarrow \tau_m} \frac{\epsilon}{k_B T} \left\langle \delta \dot{N}_b(t) \delta N_a(0) \right\rangle_0 \\
&= \lim_{t \rightarrow \tau_m} \frac{\left(\langle \rho(z_a) \rangle - \langle \rho(z_b) \rangle \right)}{\rho_0} \times \left\langle \delta \dot{N}_b(t) \delta N_a(0) \right\rangle_0 \\
&= \left(\langle \rho(z_a) \rangle - \langle \rho(z_b) \rangle \right) P
\end{aligned} \tag{2.6}$$

where the permeability coefficient is defined as,

$$P = \lim_{t \rightarrow \tau_m} \left\langle \delta \dot{N}_b(t) \delta N_a(0) \right\rangle_0 \frac{1}{\rho_0} \tag{2.7}$$

Here, the time τ_m represents the molecular timescale needed for the net flux, J , to relax to its steady state value after the short initial dynamical transient effects have subsided. The membrane coefficient, P , defined by eq 2.7 has the dimension of a velocity (length per unit of time). The flux, J (the number of molecules crossing the membrane per unit of time), can be converted into a flux density, j (the number of molecules crossing the membrane per unit of time and unit area), by dividing by the membrane surface area, A , yielding the familiar constitutive relation of membrane permeation, $j = P \Delta C$, where $\Delta C = (\langle \rho(z_a) \rangle - \langle \rho(z_b) \rangle)/A$ is the difference in the bulk concentration between regions a and b (Figure 2.1).

The time-correlation function defining the permeability coefficient involves cross-terms between different permeant molecules i and j . If the overall concentration of the permeant molecule is sufficiently low, the $i \neq j$ cross-terms between different molecules can be neglected, and the final expression only involves a sum over the time-correlation function of

single permeant molecules,

$$\begin{aligned}
P &= \lim_{t \rightarrow \tau_m} \sum_i^{N_{\text{tot}}} \left\langle \delta \dot{H}_b(\mathbf{r}_i(t)) \delta H_a(\mathbf{r}_i(0)) \right\rangle_0 \frac{1}{\rho_0} \\
&= \lim_{t \rightarrow \tau_m} \left\langle \delta \dot{H}_b(\mathbf{r}_1(t)) \delta H_a(\mathbf{r}_1(0)) \right\rangle_0 \frac{1}{\pi(z_a)}
\end{aligned} \tag{2.8}$$

where $\pi(z_a) = \langle \delta(z_1 - z_a) \rangle = \langle \rho(z_a) \rangle / N_{\text{tot}}$ is the one-dimensional equilibrium probability distribution of the permeant molecule #1 along the z -axis in the unperturbed system with the Hamiltonian $\mathcal{H}_0$. Picking any given permeant molecule to write eq 2.8 is correct because they are indistinguishable. Because $\langle \delta \dot{H}_b(t) \delta H_a(0) \rangle_0 = \langle \dot{H}_b(t) H_a(0) \rangle_0$, one can express the permeability coefficient as the limiting slope of an equilibrium time-correlation function of the indicator functions, $C_{ab}(t) = \langle H_a(0) H_b(t) \rangle_0$, normalized by the one-dimensional probability $\pi(z_a)$,

$$P = \lim_{t \rightarrow \tau_m} \dot{C}_{ab}(t) \frac{1}{\pi(z_a)} \tag{2.9}$$

This expression is reminiscent of the theoretical treatment of rare transitions between stable states, where the same time-correlation function of indicator functions $C_{ab}(t)$ is normalized by the probability of the stable states, π_a and π_b .⁶⁶ In the following, the analysis of the permeability will be limited to considerations of the dynamics of a single permeant molecule across the membrane region.

In summary, this statistical mechanical analysis shows that there is an average steady-state flux across a membrane in response to a small shift in the equilibrium concentration of a permeant molecule, obeying the familiar constitutive relation $j = P \Delta C$. The dynamics of the unperturbed system is governed by the Hamiltonian $\mathcal{H}_0$, and the permeability coefficient P is expressed in terms of an equilibrium time-correlation function of the indicator functions (eq 2.9). The analysis is general and does not make any assumption about the nature of the

microscopic dynamics of the permeant molecule as it crosses the membrane.

2.2.2 Net flux, recrossings, and transmission coefficient

As shown in Figure 2.1, the one-molecular population operators H_a and H_b correspond to two contiguous spatial regions that are touching at some dividing surface $z^\dagger$ in the membrane region. These considerations are useful to clarify the significance of the expression for the permeability coefficient as,

$$\begin{aligned}
P &= \lim_{t \rightarrow \tau_m} \dot{C}_{ab}(t) \frac{1}{\pi(z_a)} \\
&= \lim_{t \rightarrow \tau_m} \left\langle H_a(0) \dot{H}_b(t) \right\rangle \frac{1}{\pi(z_a)} \\
&= \left\langle -\dot{H}_a(0) H_b(\tau_m) \right\rangle \frac{1}{\pi(z_a)} \\
&= \left\langle \delta(z - z^\dagger) \dot{z} H_b(\tau_m) \right\rangle \frac{1}{\pi(z_a)} \\
&= \frac{\left\langle \delta(z - z^\dagger) \dot{z} H_b(\tau_m) \right\rangle}{\left\langle \delta(z - z^\dagger) \dot{z} \theta(\dot{z}) \right\rangle} \\
&\quad \times \frac{\left\langle \delta(z - z^\dagger) \dot{z} \theta(\dot{z}) \right\rangle}{\left\langle \delta(z - z^\dagger) \right\rangle} \frac{\left\langle \delta(z - z^\dagger) \right\rangle}{\pi(z_a)} \\
&= \kappa \left\langle \dot{z} \theta(\dot{z}) \right\rangle_{z^\dagger} e^{-[W(z^\dagger) - W(z_a)]/k_B T} \tag{2.10}
\end{aligned}$$

where $W(z) = -k_B T \ln \langle \rho(z) \rangle$ is a one-dimensional PMF along the z coordinate, $\langle \dot{z} \theta(\dot{z}) \rangle_{z^\dagger} = \sqrt{k_B T / 2\pi m}$ represents an approximate ideal gas-like flux for a freely-moving particle of mass m across the dividing surface $z^\dagger$ between regions a and b , and κ ,

$$\kappa = \lim_{t \rightarrow \tau_m} \frac{\left\langle \delta(z - z^\dagger) \dot{z} H_b(t) \right\rangle}{\left\langle \delta(z - z^\dagger) \dot{z} \theta(\dot{z}) \right\rangle} \tag{2.11}$$

is a correction needed to account for dynamical recrossing events that occur in the transition state region. The quantity κ is the familiar transmission coefficient arising from the theory of

activated processes, where it represents the correction between the approximate transition state theory (TST) rate and the true molecular transition rate.⁶⁶ Normally, the position of the dividing surface $z^\dagger$ would be chosen near the top of the highest free energy barrier separating regions a and b to capture the dominant rate-limiting step of the crossing event.

This analysis shows how the permeability coefficient, P , of a substance crossing a membrane can be expressed in terms of an equilibrium time-correlation function of the indicator functions, $C_{ab}(t) = \langle H_a(0)\dot{H}_b(t) \rangle$, in the limit $t \rightarrow \tau_m$. This limiting process is necessary to average over the multiple random diffusional recrossings of the membrane region during a permeation event. For this reason, the lag time, τ_m , must be sufficiently long relative to those dynamical recrossing events, in order to effectively capture the true translocation rate across the membrane. An alternative to averaging over all possible recrossing events in the trajectory, $z(t)$, is to focus deliberately on the total number of successful transitions across the membrane per unit of time.^{38;12;1;51;52} These ideas go back to alternative treatments of transition rates,^{50;73;74} and are further explored in the next section.

2.2.3 *Counting membrane crossings from reactive trajectories*

To visualize the transitions between the regions a and b , we use the concept of trajectory coloring,^{75;69} by which a long equilibrium trajectory, $z(t)$, of duration $\mathcal{T}$ switches color from red to blue whenever it touches the boundary z_b coming from z_a , and switches color from blue to red when it touches the boundary z_a coming from z_b . The result is illustrated in Figure 2.2. The total time that the trajectory is red or blue is $\mathcal{T}_a$ or $\mathcal{T}_b$, respectively, and the probability that the trajectory is red or blue is related to the equilibrium probabilities of the state a and b , $\pi_a = \mathcal{T}_a/\mathcal{T}$ and $\pi_b = \mathcal{T}_b/\mathcal{T}$, respectively. The number of genuine transitions during which a permeant molecule has crossed the membrane can be monitored from the number of times, N_{ab} , the color of the trajectory changes from red to blue during time $\mathcal{T}$. At equilibrium, the number of transitions in the reverse direction is equal in magnitude, i.e.,

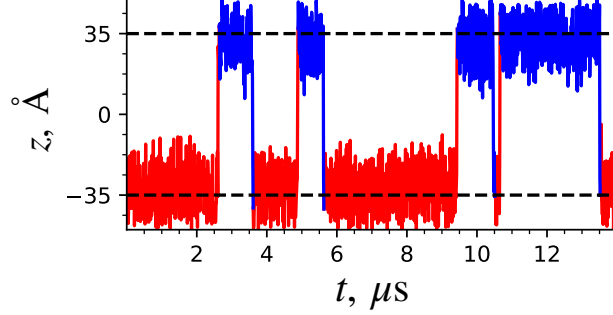


Figure 2.2: Part of a BD_e (Brownian dynamics with exponential memory) trajectory of a narrow barrier, showing color-coded crossings between a (red) and b (blue). The two regions are defined by the edge of the boundary regions $z < z_a$ for the side a and $z > z_b$ for side b , with $z_a = -35 \text{ \AA}$, and $z_b = +35 \text{ \AA}$.

$N_{ba} = N_{ab}$, yielding zero total net flux on average. One may note that trajectory coloring is insensitive to any rapid recrossing events that occur in the neighborhood of $z^\ddagger$. The unidirectional flux from state a to state b on the basis of this counting argument is,

$$J_{ab} = \lim_{\mathcal{T} \rightarrow \infty} \frac{N_{ab}}{\mathcal{T}} \quad (2.12)$$

and the permeability coefficient is

$$P = J_{ab} \frac{1}{\rho_0} \quad (2.13)$$

This is essentially the transition counting method^{50;73;74} used by Pastor and coworkers to determine the permeability coefficient from unbiased MD simulations.¹ The coloring identifies the set of reactive trajectories that defines the transition path ensemble as prescribed in TPT.¹⁷ Within the TPT framework, we can express the net forward reactive flux from the local transitions $z \rightarrow z'$ by considering the evolution of a trajectory $z(t)$ of length $\mathcal{T}$.^{67;17;69;70} If the dynamics of the system along z is assumed to be Markovian with a lag time τ , the net

flux from a to b can be written as,

$$\begin{aligned}
J_{ab} &= \lim_{\mathcal{T} \rightarrow \infty} \frac{1}{\mathcal{T}} \int_0^{\mathcal{T}} \int_{z < z_a} dz \int_{z' > z_b} dz' \mathbb{1}_{[z(t) \in R]} \\
&\quad \times \frac{\delta(z' - z(t + \tau)) \delta(z - z(t))}{\tau} \\
&= \int_{z < z_a} dz \int_{z' > z_b} dz' \langle j_{ab}^R(z', z) \rangle
\end{aligned} \tag{2.14}$$

where the indicator function $\mathbb{1}_{[z(t) \in R]} = 1$ if at time t , $z(t)$ belongs to an a -to- b “reactive” path, and is equal to 0 otherwise, and $\langle j_{ab}^R(z', z) \rangle$ represents the mean net number of transitions from z to z' per unit of time τ contributed by a to b reactive trajectories. In the double integral, region a' is defined to include all $z < z_a$, and region b' is defined to include all $z > z_b$. This expression shows that the fate of the trajectory over the lag time τ can be built directly into the analysis via the indicator function $\mathbb{1}_{[z(t) \in R]}$ to focus on the occurrence of genuine “reactive” transitions. To further develop this analysis within the TPT framework, it is necessary to introduce the probability $q(z)$ that the system starting at position z will dynamically evolve to reach z_b before reaching z_a . The probability $q(z)$, which is a key concept associated with the transition path ensemble, is called the committor.^{76;67;17;68;69;70;18}

2.2.4 Transition path theory, committor probability, and permeability

The net reactive current $\langle j_{ab}^R(z', z) \rangle$ from z to z' is obtained by summing over all reactive trajectories that actually make a transition from a to b . Thus, to determine the fate a reactive trajectory that makes a transition from z to z' , we have to consider the equilibrium probability of state z , i.e., $\pi(z)$, the probability that the piece of trajectory reaching z came from a , i.e., $(1 - q(z))$, and the probability that the piece of trajectory after leaving z' will go on to b , i.e., $q(z')$. This yields $q(z') \mathcal{P}_\tau(z'|z) (1 - q(z)) \pi(z)$, where $\mathcal{P}_\tau(z'|z)$ is the propagator of the system (transfer operator) for the continuous step $z \rightarrow z'$, which is assumed to be Markovian for the lag time τ . The propagator obeys microscopic detailed balance,

$\mathcal{P}_\tau(z|z') \pi(z') = \mathcal{P}_\tau(z'|z) \pi(z)$. To obtain the net flux from z to z' , we have to subtract the reverse reactive transition from z' to z , by considering the equilibrium probability of state z' , i.e., $\pi(z')$, the probability that the piece of trajectory reaching z' came from a , i.e., $(1 - q(z))$, and the probability that the piece of trajectory after leaving z will lead to b , i.e., $q(z)$. This yields, $q(z) \mathcal{P}_\tau(z|z') (1 - q(z')) \pi(z')$. The resulting net probability current of the reactive trajectories between z and z' is thus,

$$\begin{aligned} \left\langle j_{ab}^R(z', z) \right\rangle \tau &= q(z') \mathcal{P}_\tau(z'|z) (1 - q(z)) \pi(z) \\ &\quad - q(z) \mathcal{P}_\tau(z|z') (1 - q(z')) \pi(z') \end{aligned} \quad (2.15)$$

which can, using microscopic detailed balance, be written as

$$\left\langle j_{ab}^R(z', z) \right\rangle \tau = \left(q(z') - q(z) \right) \mathcal{P}_\tau(z'|z) \pi(z) \quad (2.16)$$

The net flux can then be written as,¹⁸

$$\begin{aligned} J_{ab} &= \frac{1}{\tau} \int_{z \in a'} dz \int_{z' \in b'} dz' \left(q(z') - q(z) \right) \\ &\quad \times \mathcal{P}_\tau(z'|z) \pi(z) \end{aligned} \quad (2.17)$$

and additional considerations lead to the form,¹⁸

$$\begin{aligned} J_{ab} &= \frac{1}{2\tau} \int dz \int dz' \left(q(z') - q(z) \right)^2 \\ &\quad \times \mathcal{P}_\tau(z'|z) \pi(z) \\ &= \frac{1}{2\tau} \langle (q(\tau) - q(0))^2 \rangle \\ &= \frac{1}{\tau} C_{qq}(t) \end{aligned} \quad (2.18)$$

where $C_{qq}(t) = \frac{1}{2}\langle (q(t)-q(0))^2 \rangle = (\langle q(0)q(0) \rangle - \langle q(t)q(0) \rangle)$ is the committor time-correlation function. One can show that J_{ab} corresponds to the limiting slope of $C_{qq}(t)$,¹⁸ such that the permeability coefficient may be written as,

$$P = \lim_{t \rightarrow \tau} \dot{C}_{qq}(t) \frac{1}{\pi(z_a)} \quad (2.19)$$

For a purely overdamped diffusive system, the dynamics is Markovian in the limit of $t \rightarrow 0^+$.

In this case, the committor is exactly given by,^{76;18}

$$q(z) = \frac{\int_{z_a}^z dz' e^{W(z')/k_B T} / D(z')}{\int_{z_a}^{z_b} dz' e^{W(z')/k_B T} / D(z')} \quad (2.20)$$

where the permeability coefficient is given as

$$P = \lim_{t \rightarrow 0^+} \dot{C}_{qq}(t) \frac{1}{\pi(z_a)} \quad (2.21)$$

Following the assumption of a purely diffusive overdamped dynamics, the limit $\lim_{t \rightarrow 0^+} \dot{C}_{qq}(t)$ can, after a little algebra, be written as $D\langle (dq/dz)^2 \rangle$ to recover the familiar ISD expression of eq 2.1.¹⁸

However, when there are memory effects and the evolution of the system is not purely diffusive, eq 2.20 does not yield the exact committor. The expression can still be used to extract the exact permeability coefficient by using an appropriate lag time, τ , for which the dynamics becomes Markovian. It is sufficient to observe that the slope of the committor time-correlation function converges to a stable value. Accordingly, the permeability coefficient is given by eq 2.19, where the limit must be taken to the appropriate value of τ that renders the dynamics Markovian. In this context, even though the expression of eq 2.20 only provides an approximation of the true committor, it can serve as a sound basis to accurately determine

the exact permeability coefficient in the limit of a lag time τ that recovers markovianity. This remarkable observation can be explained via a spectral analysis of the eigenmodes of the propagator.^{70;18}

2.2.5 Generalized Langevin equation and correction to the ISD model

It is of interest to test the validity of the different theoretical and computational routes for the determination of the permeability coefficient presented by eq 2.9, eq 2.10, and eq 2.19. For this purpose, a useful model of the dynamics of a permeant molecule in a membrane is provided by the generalized Langevin equation (GLE),^{71;14;72}

$$m\ddot{z} = F(z) - \int_0^t M(t') \dot{z}(t-t') dt' + f(t) \quad (2.22)$$

where $F(z) = -W'(z)$ is the mean force based on the PMF of the permeant molecule across the membrane, $M(t)$ is the memory function, and $f(t)$ is the random force obeying the fluctuation-dissipation theorem, $\langle f(t)f(0) \rangle = k_B T M(t)$. For a harmonic functional form, the potential is $W(z) = \frac{1}{2}(W'')z^2$, and the mean force is $F = -W'' z$. For a harmonic well, $W'' > 0$, whereas $W'' < 0$ for an inverted harmonic barrier. The following development is valid for both a harmonic well or barrier, but we will sometimes write W'' with an explicit absolute value to avoid any sign ambiguity. Using the Laplace transform of the GLE, we write,^{77;9}

$$\begin{aligned} -v_0 + s \left(-z_0 + s\tilde{z}(s) \right) &= -\frac{W''}{m} \tilde{z}(s) - \frac{\tilde{M}(s)}{m} \left(-z_0 + s\tilde{z}(s) \right) + \frac{\tilde{f}(s)}{m} \\ \tilde{z}(s) &= z_0 \frac{s + \tilde{M}(s)/m}{\Delta(s)} + v_0 \frac{1}{\Delta(s)} + \frac{\tilde{f}(s)}{m} \frac{1}{\Delta(s)} \\ &= z_0 \tilde{C}_{11}(s) + v_0 \tilde{C}_{12}(s) + \frac{\tilde{f}(s)}{m} \frac{1}{\Delta(s)} \end{aligned} \quad (2.23)$$

where $\Delta(s) = (W''/m + s^2 + s\widetilde{M}(s)/m)$. Pursuing this analysis makes it possible to determine various properties, including the mean squared deviation (MSD) of displacements in closed form.^{77;9} For the sake of simplicity, we will assume that the memory function $M(t)$ comprises a Markovian instantaneous Einstein-Smoluchowski friction along z , $\gamma_z \delta(t)$, superimposed on a decaying exponential memory term with amplitude K_e and relaxation time τ_e ,

$$M(t) = \gamma_z \delta(t) + K_e e^{-t/\tau_e} \quad (2.24)$$

The total friction component arising from the exponential memory is $\gamma_e = K_e \tau_e$. The Laplace transform of the exponential memory function is,

$$\begin{aligned} \widetilde{M}(s) &= \int_0^\infty e^{-st} M(t) dt \\ &= \gamma_z + \frac{K_e}{s + 1/\tau_e} \end{aligned} \quad (2.25)$$

Assuming that memory effects have fully relaxed, $\lim_{s \rightarrow 0} \widetilde{M}(s)$, yields the total effective friction, $\gamma_{\text{eff}} = \gamma_z + \gamma_e$, which corresponds to an effective diffusion coefficient using the Einstein relation, $D_{\text{eff}} = k_B T / \gamma_{\text{eff}}$.

Let us first examine the issue of the diffusion coefficient, which plays a central role in the ISD treatment. In fact, this treatment is an approximation that relies on the existence of an effective diffusion coefficient, D_{eff} . In the context of all-atom simulations, this is typically determined by the evolution of the MSD of the molecule over a short lag time. Further analysis, however, will show that this route does not always lead to consistent results (see below). Using the GLE with the simple exponential memory function (eq 2.24), we examine whether the diffusivity can be extracted reliably from the MSD for overdamped dynamics. Based on the solution of the overdamped GLE with a harmonic potential,^{77;9} the variance

of a particle's position (effectively the MSD) can be expressed as,

$$\langle z^2(t) \rangle - \langle z(t) \rangle^2 = \frac{k_B T}{W''} \left(1 - (C_{11}(t))^2 \right) \quad (2.26)$$

where C_{11} is a known function defined from eq 2.23.^{77;9} For the overdamped dynamics with exponential memory function (eq 2.24), the MSD is,

$$C_{11}(t) = \frac{1}{s_- - s_+} \left[(s_- + 1/\tau_e + K_e/\gamma_z) e^{ts_-} - (s_+ + 1/\tau_e + K_e/\gamma_z) e^{ts_+} \right] \quad (2.27)$$

where s_+ and s_- are the positive and negative roots solutions, respectively, from eq 2.30, which depend on the parameters of the memory function and the curvature of the barrier. A complete derivation of the analytical MSD expression is provided in Appendix A.

We now return to eq 2.10 to derive a form of the permeability coefficient that goes beyond the ISD approximation by directly accounting for memory effects. The analysis will lead to a non-Markovian corrective factor to the traditional ISD permeability of an inverted harmonic barrier. According to Grote-Hynes theory,¹⁶ the transmission coefficient, κ , is typically written as s/ω_0 , where $\omega_0 = \sqrt{-W''/m}$ is the effective frequency of the barrier, and s is the solution of the equation involving the Laplace transform of the memory function,

$$\omega_0^2 - s^2 = \frac{s}{m} \widetilde{M}(s) \quad (2.28)$$

Note that for an inverted harmonic barrier, $W'' < 0$. In the overdamped limit of $m \rightarrow 0$ (neglecting the inertial term ms^2), eq 2.28 becomes,

$$-W'' = s \widetilde{M}(s) \quad (2.29)$$

The solutions of eq 2.29 are,

$$s_{\pm} = \frac{1}{2\gamma_z\tau_e} \left[- \left(\gamma_z + K_e\tau_e + W''\tau_e \right) \pm \sqrt{\left(\gamma_z + K_e\tau_e + W''\tau_e \right)^2 - 4W''\gamma_z\tau_e} \right] \quad (2.30)$$

For the transmission coefficient of Grote-Hynes theory, we only consider a barrier with negative curvature. To avoid sign ambiguity, we express the positive solution of eq 2.29 in terms of the absolute value of the negative curvature, $|W''|$,

$$s_+ \approx \frac{|W''|}{\gamma_z + K_e\tau_e} \left(\frac{1}{1 - |W''|\tau_e/(\gamma_z + K_e\tau_e)} \right) \quad (2.31)$$

which corresponds to an approximate transmission coefficient of,

$$\kappa \approx \sqrt{m|W''|} \frac{D_{\text{eff}}}{k_B T} \left(\frac{1}{1 - |W''|D_{\text{eff}}\tau_e/k_B T} \right) \quad (2.32)$$

The permeability coefficient following eq 2.10 is,

$$\begin{aligned} P &\approx D_{\text{eff}} \sqrt{\frac{|W''|}{k_B T 2\pi}} e^{-[W(z^\dagger) - W(z_a)]/k_B T} \\ &\quad \left(\frac{1}{1 - |W''|D_{\text{eff}}\tau_e/k_B T} \right) \\ &\approx P_{\text{ISD}} \left(\frac{1}{1 - \tau_e/\tau_0} \right) \end{aligned} \quad (2.33)$$

where $\tau_0 = k_B T/|W''|D_{\text{eff}}$ represents the inherent relaxation time associated with the dynamics across the inverted harmonic barrier. Here, P_{ISD} is the classic ISD permeability coefficient from eq 2.1 for a harmonic barrier of curvature $|W''|$ with $D_{\text{eff}} = k_B T/\gamma_{\text{eff}}$. The

ISD approximation is recovered in the limit of $\tau_e \ll \tau_0$, when the timescale from the memory is much shorter than the timescale associated with dynamics across the barrier. The permeability coefficient, P , becomes larger than P_{ISD} as τ_e/τ_0 increases. More details about the full derivation are given in section S2 of the SI.

2.3 Simulations and Methods

2.3.1 Simulation of the overdamped GLE

Ignoring inertial effects ($m\ddot{z} \approx 0$), the GLE of eq A.1 with the exponential memory of eq 2.24 can be simulated as a set of two coupled Brownian dynamics (BD) equations,

$$\begin{aligned} z(t + \Delta t) &= z(t) \\ &- \frac{D_z}{k_B T} \left(\frac{\partial W(z)}{\partial z} + K_e(z - z_e) \right) \Delta t \\ &+ g_z \end{aligned} \quad (2.34)$$

Table 2.1: Summary of BD and BD_e simulations

description	barrier	K_e ($\text{\AA}^2$)	(kcal/mol	D_e ($\text{\AA}^2/\text{ps}$)	replicates	time/rep	total (μs)	time
MSD	none	0.05		0.1	10^6	500 ps	500	
MSD	none	0.05		0.2	10^6	500 ps	500	
MSD	none	0.10		0.1	10^6	500 ps	500	
Grote-Hynes	parabolic	0.4		0.01	50	50 μs	2500	
committor/ counting	narrow	0.05		0.1	50	50 μs	2500	
committor/ counting	flat-top	0.05		0.1	50	50 μs	2500	
committor/ counting	narrow	0.0	–	–	50	50 μs	2500	
committor/ counting	flat-top	0.0	–	–	50	50 μs	2500	
committor “short traj”	narrow	0.05		0.1	2.5×10^6	500 ps	1250	
committor “short traj”	flat-top	0.05		0.1	2.5×10^6	500 ps	1250	

and

$$z_e(t + \Delta t) = z_e(t) + \frac{D_e}{k_B T} K_e (z - z_e) \Delta t + g_e \quad (2.35)$$

where g_z and g_e are uncorrelated Gaussian random numbers with zero average and mean quadratic deviations equal to $2D_z\Delta t$ and $2D_e\Delta t$, respectively. The diffusion constant, D_z , is related to the direct Markovian friction along the z coordinate, $D_z = k_B T / \gamma_z$. In practice, eq 2.34 and eq 2.35 essentially represent a BD algorithm with two degrees of freedom that recapitulate the overdamped dynamics of the permeant molecule along the z -axis with an exponential memory and no inertial effects.⁷⁸

It can be shown that integrating $z_e(t)$ explicitly and reinserting it into the equation of motion of $z(t)$ returns the overdamped form of the GLE with exponential memory, $M(t) = \gamma_z \delta(t) + K_e e^{-t/\tau_e}$, and with the associated random force obeying the fluctuation-dissipation theorem. We will refer to this set of equations as BD_e . We also consider corresponding BD simulations, which are equivalent to their BD_e counterparts, with the exception that K_e is set to zero (and z is not coupled to z_e). The BD and BD_e equations were integrated with a timestep of 10 fs. Reflecting boundary conditions were set at $z = \pm 50$ Å.

To illustrate the effects of memory, two model PMFs corresponding to a “narrow” and a broad “flat-top” free energy barrier were considered, with $W^{\text{narrow}}(z) = 3.6531 \exp[-(z/0.002828)^2]$ and $W^{\text{flat-top}}(z) = 3 \exp[-(z/0.006132)^8]$. The model PMFs were designed with slightly different free-energy barrier maximum heights such that they have the same permeability according to eq 2.1. It is therefore possible to probe the effect of barrier curvature on permeability with and without memory by extracting the exact values from the BD and BD_e simulations. The set of BD and BD_e simulations, including the extent of sampling and parameters of the memory function, is summarized in Table 2.1.

2.3.2 Water permeation

Unbiased simulations for counting and committor correlation permeability. Unbiased MD simulations of a small 1,2-dilauroyl-*sn*-glycero-3-phosphocholine (DLPC) bilayer in water at 303 K were performed to compare the counting permeability and committor correlation permeability to water. The simulations were run in three replicates, each starting from a different configuration, which was pre-equilibrated with the CHARMM36 force field, and then converted to the Drude representation with Drude Prepper.⁷⁹ Each system consisted of 2880 SWM4 water molecules⁸⁰ and 72 DLPC lipids modeled with the Drude2023 force field.^{81;30} The MD simulations were run with OpenMM version 7.7⁸² and the rickflow⁵² front-end, using the Drude Nose-Hoover integrator with a timestep of 1 fs, a friction of 10 ps⁻¹, and a chain length, number of steps, and Yoshida-Suzuki all set to 3. The Drude particles were given a temperature of 1 K and a friction of 1 ps⁻¹. LJ-PME was used for non-bonded interactions with a cutoff of 10 Å. Periodic boundary conditions were used.

Each system was equilibrated at a constant pressure of 1 atm for 100 ns using the Monte Carlo membrane barostat. An initial configuration for each replicate was then selected by the criteria that its volume and surface area approximately equal those of the ensemble average. The dimensions for x , y , and z were found to be $47.3 \times 47.3 \times 70.8$ Å (where $x \times y$ is the surface area of one membrane leaflet). These configurations were then simulated at constant volume for 400 ns each for a total of 1.2 μ s to compute the PMF, the committor time-correlation function, and the counting permeability. Technically, the frames were saved every 5 ps for accurate counting of crossing events. The z -components of the distances between each water oxygen and the center of mass of the membrane were unwrapped for each trajectory, using the known constant z -dimension of the box. This definition of z was used to determine the PMF, to compute the committor time-correlation function, and to count crossing events.

Umbrella sampling for position-dependent diffusivity. In order to obtain an

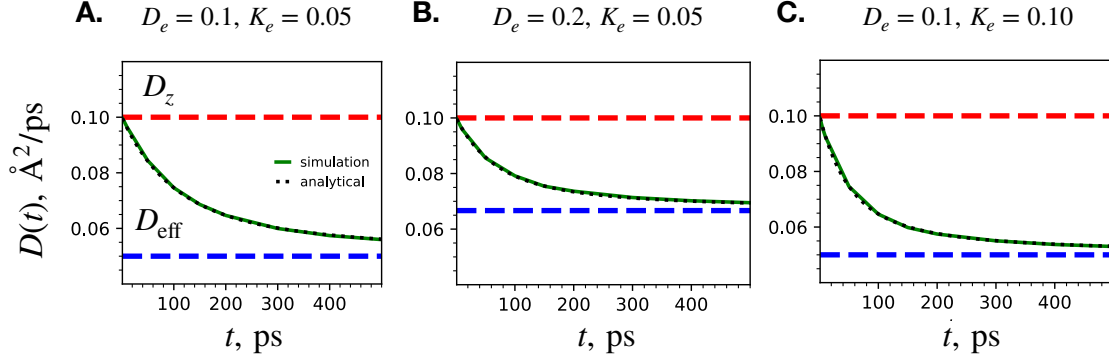


Figure 2.3: MSD diffusivity computed by BD_e simulations (solid green line) on a flat free energy landscape ($W(z) = 0$) with various values of K_e and D_e . In each case, D_z is $0.1 \text{ Å}^2/\text{ps}$. The corresponding analytical diffusivity computed with eq 2.26 is shown for comparison (dotted black line). The limits D_z (dashed red line) and D_{eff} (dashed blue line) are shown. **A.** $D_e = 0.1 \text{ Å}^2/\text{ps}$, $K_e = 0.05 \text{ kcal/mol Å}^2$, **B.** $D_e = 0.2 \text{ Å}^2/\text{ps}$, $K_e = 0.05 \text{ kcal/mol Å}^2$, **C.** $D_e = 0.1 \text{ Å}^2/\text{ps}$, $K_e = 0.10 \text{ kcal/mol Å}^2$.

estimate of the ISD permeability of DLPC to water, umbrella sampling (US) simulations were conducted to calculate the position-dependent diffusivity of water in the membrane. The same MD protocol, force field, and system as described for the unbiased simulations were used. The US windows incorporate a harmonic potential to restrain the z -component of the center of mass distance between the water oxygen and the lipid phosphorus atoms, and were centered at 1 Å intervals from $z = -30 \text{ Å}$ to $z = +30 \text{ Å}$. The force constant was set to 10 kcal/mol Å^2 , which is not expected to significantly increase the total friction.⁸³ The initial configurations for each window were snapshots from one of the unbiased simulations, and were saved at a 1 Å interval following the crossing of a single water molecule. Each window was simulated for 30 ns , and the first 10 ns , assumed to correspond to thermalization, were excluded from the analysis. The value of z was saved every timestep (1 fs). The diffusivity along the z -coordinate was estimated based on the position autocorrelation function in each harmonic potential.^{84;5} The implementation of this method for computing the position-dependent diffusivity is discussed further in section S4 of the SI.

2.4 Results and discussion

2.4.1 MSD on a flat free-energy landscape

In subsection e) of the Theoretical Developments, we derived an analytical expression for the MSD of a particle that obeys the dynamics of the overdamped GLE with an exponential memory in a harmonic potential (eq 2.26). Here, we validate the expression by computing the MSD diffusivity numerically through BD_e simulations with various values of K_e and D_e using eq 2.34 and eq 2.35.

In each case, the curvature of the barrier (W'') along z is set to effectively zero. As shown in Figure 2.3, the MSD diffusivity begins at a maximum value that corresponds to the instantaneous diffusivity along z (D_z), and decays to the minimum effective diffusivity (D_{eff}). This behavior is qualitatively similar to what we previously observed in the physically comparable system of biased MD simulations of nicotine in a membrane with a flattened free-energy surface along z .²⁶ The analytical MSD and the BD_e simulations are in good agreement, indicating that BD_e is an effective way to numerically solve the overdamped GLE with exponential memory. The decay of the MSD diffusivity to a plateau value on a flat free-energy surface is evidence that the exponential memory function will be able to qualitatively reproduce the memory effects associated with MD simulations of small molecules' diffusing in a membrane.

As shown in Figure 2.4, the curvature ($W'' \neq 0$) greatly alters the behavior of the MSD as a function of lag time. While the MSD correctly converges to the diffusive limit in a homogeneous system with a flat free-energy profile (see Figure 2.3), the estimate of D based on the MSD is shown to rapidly deviate from any reasonable plateau when there is a well or a barrier. If the underlying dynamics is purely diffusive, as in BD, it may still be possible to extract the true diffusivity with a very short lag time. When there are memory effects, however, the short lag time MSD does not account for the full decay of the memory function.

In this case, the comparable timescale of the memory function and the timescale for crossing the free-energy landscape (barrier or well) prevents the accurate estimation the diffusivity through the MSD.

2.4.2 Grote-Hynes correction to the ISD permeability

Following the demonstration of the MSD diffusivity for the overdamped GLE with exponential memory, we propose a system for which the permeability can be solved analytically. With exponential memory and an inverted harmonic barrier along z , the permeability can be determined exactly within the framework of Grote-Hynes theory.¹⁶ The analytical expression is based on the ISD permeability, which is multiplied by the corrective factor of the transmission coefficient from Grote-Hynes (eq A.55).

In the demonstration presented here, the parameters of the memory function (D_z , D_e , K_e) and the curvature of the inverted harmonic barrier (W'') were chosen such that the pure ISD permeability would underestimate the exact analytical permeability by approximately a factor of 2 (see caption of Figure 2.5 for values). The parabolic barrier and the time-dependence of the MSD diffusivity are shown in Figure 2.5. The permeability coefficients of

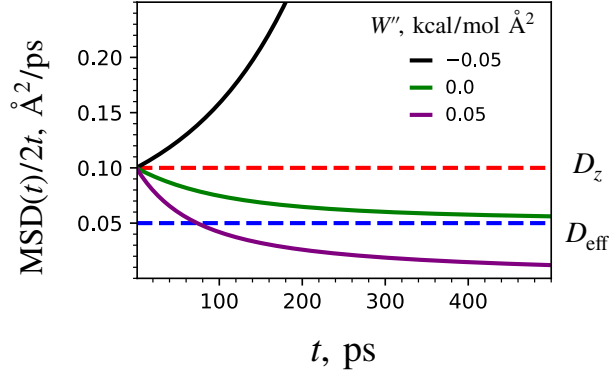


Figure 2.4: Effective MSD diffusivity as a function of the lag time computed analytically from eq 2.26 with an exponential memory for different barriers and, therefore, dynamical regimes. In each case, the instantaneous diffusivity (D_z) is set to $0.1 \text{ Å}^2/\text{ps}$ (red dashed line), the minimum effective diffusivity is $0.05 \text{ Å}^2/\text{ps}$ (blue dashed line), and the memory constant (K_e) is $0.05 \text{ kcal/mol Å}^2$. A barrier with a negative value of W'' corresponds to an inverted parabola (black line) and a positive value corresponds to a harmonic well (purple line), while zero is a flat landscape (green line).

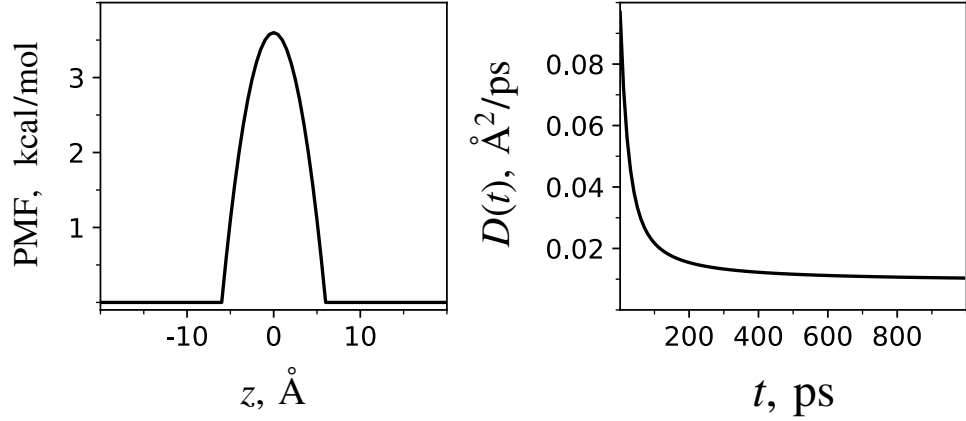


Figure 2.5: PMF, $W(z)$, (left) and analytical MSD diffusivity (right) for demonstrating the Grote-Hynes correction to the ISD permeability of an inverted harmonic barrier with exponential memory. The PMF has a curvature $|W''| = 0.2 \text{ kcal/mol } \text{\AA}^2$, and it reaches 0 where $z = \pm 6$. The MSD corresponds to parameters: $D_z = 0.1$, $D_e = 0.01 \text{ } \text{\AA}^2/\text{ps}$, $K_e = 0.4 \text{ kcal/mol } \text{\AA}^2$.

Table 2.2: Calculated permeability for inverted harmonic barrier with exponential memory

Method		$P \text{ (cm/s)}$
ISD	eq 2.1	0.058
G-H (exact)	eq 2.30	0.094
G-H (approx.)	eq 2.32	0.11
Counting	eq 2.13	0.098 ± 0.034

Notes: Counting permeability based on ensemble average and standard deviation of 50 replicates of $50 \mu\text{s}$ each of BD_e simulations. The mean and standard deviation of total crossings per replicate are 10.88 ± 3.8 .

this system, as determined by several methods, are summarized in Table 2.2. The analytical ISD permeability of a parabolic barrier with exponential memory is given by eq A.55, showing that the approximate transmission coefficient has the form $1/(1 - \tau_e/\tau_0)$, where $\tau_e = k_B T / K_e D_e = 153 \text{ ps}$ and $\tau_0 = k_B T / |W''| D_{\text{eff}} = 337 \text{ ps}$. It follows that the corrective factor, which accounts for the exponential memory and curvature of the free-energy barrier, is approximately 1.83. As a validation of the exact analytical permeability coefficient for this system, we used BD_e simulations to compute the flux across the barrier directly by counting crossing events (eq 2.13).

As shown in Table 2.2, the exact analytical Grote-Hynes permeability is in very good

agreement with the simulation, and the approximate analytical permeability is within the uncertainty interval of the simulation. We note that the approximation of the corrective factor based on eq 2.31 is expected to approach the exact corrective factor (eq 2.30) as the ratio of $K_e/|W''|$ increases. For example, when $|W''|$ is 0.2 kcal/mol $\text{\AA}^2$ and K_e is 0.4 kcal/mol $\text{\AA}^2$, the ratio $s_{\text{approx}}/s_{\text{exact}}$ is about 1.12. If K_e is doubled, the ratio will reduce to 1.03.

While this analysis is only exact for the overdamped GLE with an exponential memory and an inverted harmonic barrier, it clarifies which characteristics of the system lead to deviations from the pure ISD model. The transmission coefficient increases as the ratio τ_e/τ_0 increases, which can be expressed in terms of the parameters of the system as $(|W''|/K_e)(D_z/(D_z + D_e))$. The transmission coefficient, therefore, increases from a larger barrier curvature ($|W''|$), larger D_z , smaller K_e , and smaller D_e . The combination of Grote-Hynes theory with the ISD model illustrates how the permeability coefficient is affected by the diffusivity estimated from the MSD and the timescale of the barrier crossing.

The total effective friction is not felt by the molecule as it crosses the top of the free-energy barrier because the timescale of the memory function is comparable to the timescale for traveling across the barrier. Thus, the effect of memory on a curved free-energy landscape yields a smaller effective friction and a larger permeability coefficient than that estimated by the ISD approximation based on the total effective friction. Ultimately, these observations point to the main issue at the heart of this approximation, which is the assumption of memory-less diffusive dynamics.

2.4.3 Permeability from BD simulations with memory effects

The ability of BD_e with harmonically coupled z and z_e coordinates to numerically solve for permeability can be extended to arbitrary barriers. As outlined in subsection a) of the Methods, two barriers are constructed as test systems for characterizing the effect of memory

on permeability. The narrow barrier is a Gaussian with a curvature at its peak. The flat-top barrier is constructed such that it has a second derivative of zero at its peak. Additionally, the precise heights of the barriers are chosen to give each system the same permeability within the ISD framework, assuming the same D_{eff} (position-independent minimum effective diffusivity). The deviations in the exact permeability between each system can, therefore, be attributed to the interplay of the exponential memory and the curvature of each barrier. Here, we show the permeability of each barrier computed with the pure ISD model (eq 2.1), and compare it to the BD_e simulations, which are interpreted in terms of counting crossing events (eq 2.13), as well as the committor time-correlation function (eq 2.19). For reference, the permeability of BD simulations of equivalent barriers without memory effects are also reported.

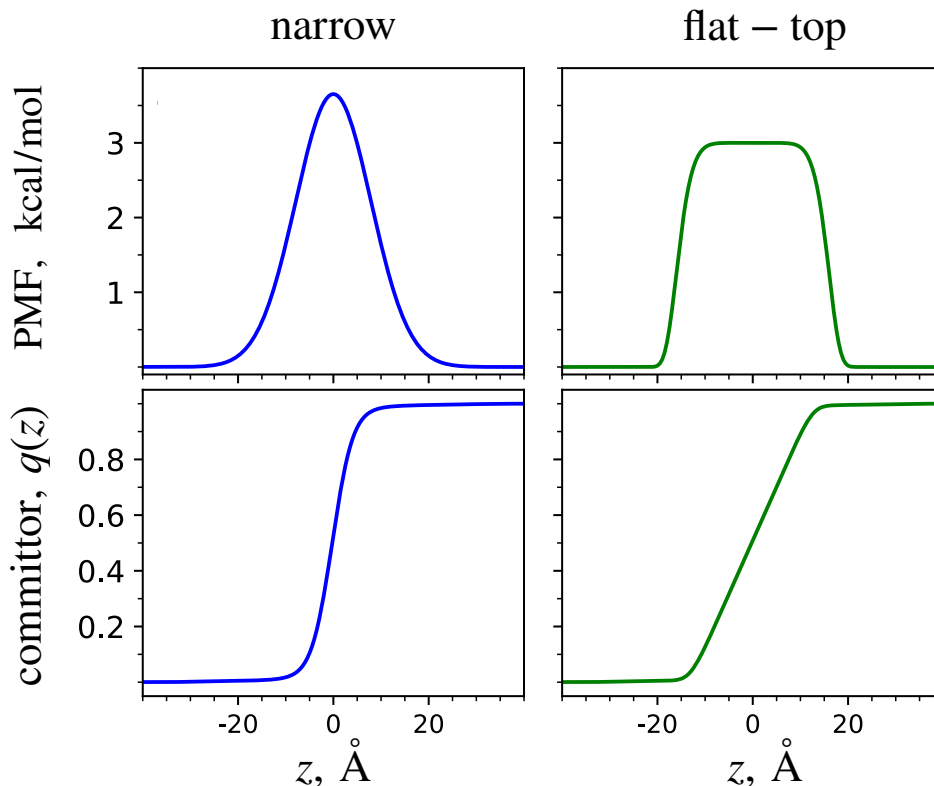


Figure 2.6: PMF, $W(z)$, and approximate committor, $q(z)$, for the narrow (left side) and flat-top (right side) barriers used in the BD_e and BD simulations.

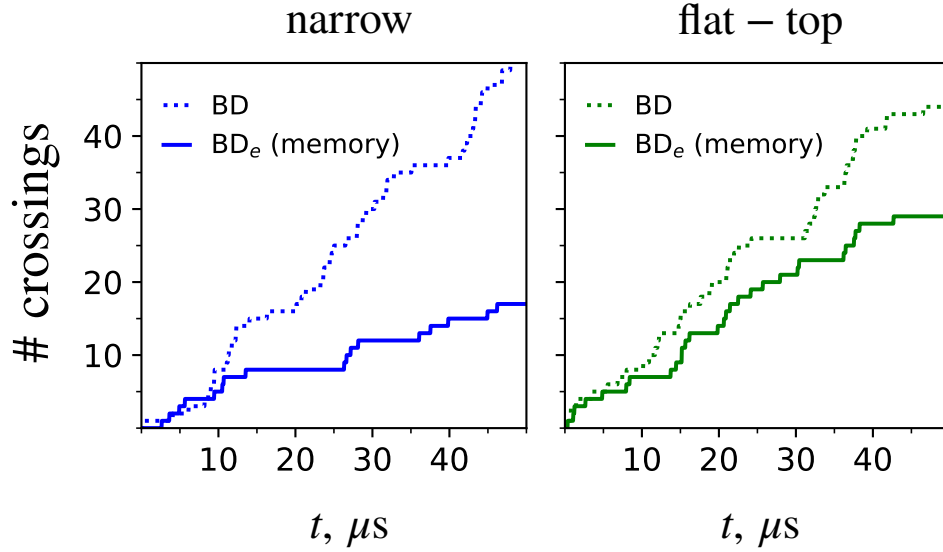


Figure 2.7: Total number of crossing events from BD_e (solid lines) and BD (dashed lines) simulations of each barrier (narrow = blue, flat-top = green). The mean and standard deviation of total crossings per replicate from ensembles of 50 replicates of 50 μs each are 42.12 ± 6.4 for the BD narrow, 27.20 ± 4.9 for the BD_e narrow, 43.94 ± 6.5 for the BD flat-top, and 24.48 ± 5.6 for the BD_e flat-top.

The PMFs for each system are depicted in Figure 2.6. Also shown are the approximate committors, which are computed with eq 2.20. The approximate committor for the narrow barrier has a steep slope, which would approach a step function as the curvature increases. The committor for the flat-top barrier has an approximately linear increase. The results of one of each type of the BD_e simulations in terms of the total number of crossings counted over time are gathered in Figure 2.7. It is immediately evident that the memoryless systems have significantly more crossings, due to the absence of the exponential memory terms K_e and D_e , which results in a D_{eff} value double that of the systems with memory.

The permeability results for each set of simulations are gathered in Table 2.3. In each case, the pure ISD permeability is computed with D_z for BD and D_{eff} for BD_e . The ISD permeability values are equal for each barrier by construction. In practice, the permeability coefficients estimated from the committor via eq 2.19 were obtained by first calculating the time-correlation function (eq 2.18) from the trajectory using (eq 2.20) for $q(z)$, and then fitting the time-correlation function to the form, $C_{qq}(t) \approx -ae^{-bt} + mt + d$, where the

Table 2.3: Permeability from BD simulations with memory effects (cm/s)

barrier	dynamics	ISD (eq 2.1)	counting (eq 2.13)	committor (eq 2.19)
narrow	BD	0.29	0.28	0.28
flat-top	BD	0.29	0.29	0.29
narrow	BD _e	0.14	0.18	0.18
flat-top	BD _e	0.14	0.16	0.16

Notes: BD corresponds to 1D (memoryless) BD, and BD_e corresponds to BD with an exponential memory. The reported ISD permeability corresponds to the analytical minimum D_{eff} , or $\frac{1}{1/D_z + 1/D_e}$, and the committor permeability corresponds to the respective plateau value of Figure 2.8. The uncertainty of the counting values is on the order of 0.03–0.04.

exponential and constant term account for short lag time transient behavior due to the use of the approximate committor and non-ideal reaction coordinate model.¹⁸ The permeability coefficient is determined by the constant m , which is proportional to the finite plateau value of eq 2.19. The fitting was done using SciPy’s curve-fitting function.⁸⁵

The committor time-correlation functions computed with eq 2.18 and the appropriate approximate committor from the $z(t)$ trajectory data for each set of simulations are shown in the top row of Figure 2.8. Also shown are the corresponding Heaviside time-correlation functions. The steepness of the long lag time slope of these time-correlation functions is directly proportional to the permeability of the system (eq 2.19).

In the second row of Figure 2.8, the permeability computed with each method as a function of the lag time is plotted for the BD_e systems. In each case, the counting permeability is lag-time-independent because it is determined by the total number of crossing events and the total simulation time. In addition, for both barriers, the plateau value of the committor permeability agrees very well with the counting permeability. For the ISD permeability, however, the agreement with counting depends on the barrier. The minimum ISD permeability of the narrow barrier is considerably smaller than the corresponding counting and committor permeability, which is evident visually from Figure 2.8, or roughly a factor of 1.28 times smaller, referring to Table 2.3. The deviation is less significant for the flat-top barrier,

for which the ISD permeability is only a factor of 1.13 times smaller than the counting and committor permeability.

The Heaviside permeability requires a longer lag time to converge to the same plateau as the committor and counting permeability. The lag time is longer for the flat-top barrier because the corresponding committor has a more gentle slope compared to the narrow barrier, which approaches the steepness of a step function. The Heaviside function permeability for each system up to a lag time of 2000 ps is shown in section S3 of the SI

Furthermore, to illustrate the possibility of performing enhanced sampling with the com-

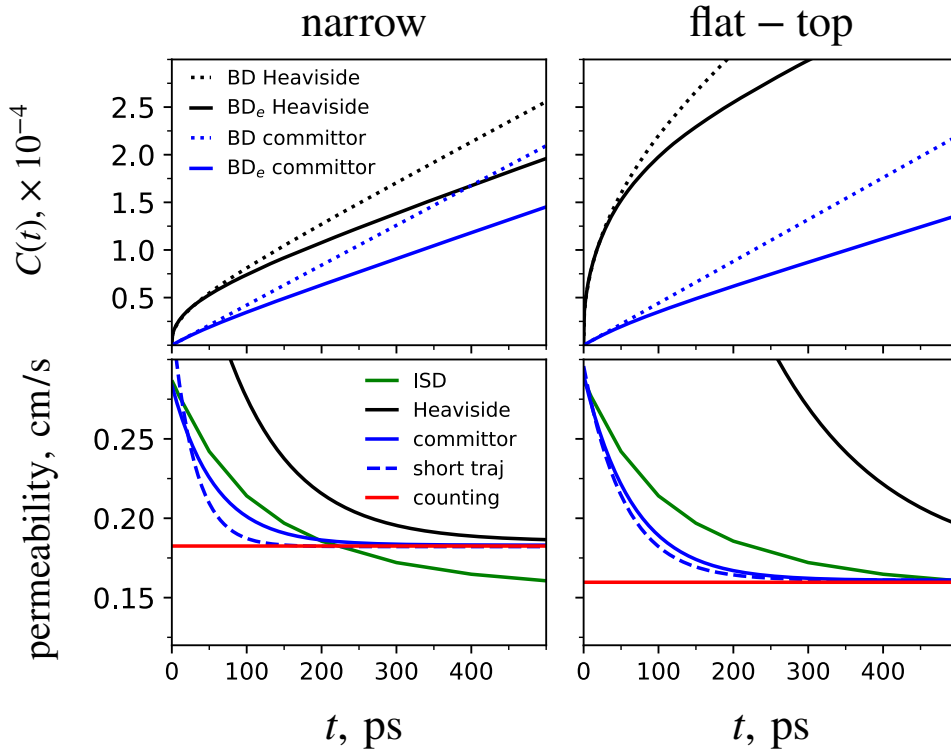


Figure 2.8: Top row: Heaviside function ($C_{ab}(t)$, black) and committor time-correlation function ($C_{qq}(t)$, blue) for BD (dotted) and BD_e (solid) simulations of the narrow (left) and flat-top (right) barriers. Bottom row: Permeability over a range of lag times for the BD_e simulations only. Permeability corresponding to the ISD model (green) computed with eq 2.1, “Heaviside” (black) with eq 2.9, “committor” (blue) with eq 2.19, “short traj” (dashed blue) with eq 2.36, and “counting” (red) with eq 2.13. The counting permeability does not depend on the lag time. The ISD permeability is the same for each barrier by construction. The permeability determined from the Heaviside time-correlation function reaches approximately the same plateau after 2000 – 3000 ps (shown in section S3 of the SI).

mittor time-correlation function $C_{qq}(t)$ from eq 2.18, also shown in Figure 2.8 is the result of a calculation labeled “short-traj”. Here, $C_{qq}(t)$ is determined from a set of short BD_e trajectories initiated randomly according to a uniform distribution $\pi(z)$ along the z -axis. The harmonic orthogonal coordinate z_e was picked from a Gaussian distribution. The contributions from the short trajectories initiated at $z_i(0)$ are re-weighted by $\pi(z_i(0))$ according to,

$$C_{qq}(t) = \frac{1}{2} \sum_{i=1}^m \frac{\pi(z_i(0))}{\sum_{j=1}^m \pi(z_j(0))} (q(z_i(t)) - q(z_i(0)))^2 \quad (2.36)$$

This type of analysis requires knowledge of the PMF along z . The “short-traj” method obtains the same plateau value of the permeability as counting crossing events and the equilibrium committor method.

We interpret the results of the BD_e simulations for a barriers with and without curvature and an exponential memory similarly to those of the parabolic barrier and the Grote-Hynes analytical permeability. Again, it is apparent that the curvature of the barrier impacts the magnitude of the deviation of the actual permeability from the ISD model. In the context of the GLE, the instantaneous diffusivity, memory function, and barrier along z are convoluted such that the exact permeability cannot be expressed analytically. For the flat-top barrier, the ISD permeability constitutes a reasonable approximation. This can be understood by analogy to the results of Figure 2.3. Due to the lack of curvature at the top of the barrier, the fully relaxed MSD is representative of the effective diffusivity. However, for the narrow barrier, the permeant molecule crosses the high curvature region quickly, without experiencing the total effective friction, leading to the permeability that is larger than that predicted by the ISD approximation. The committor time-correlation function is able to ascertain the true permeability by reconciling the timescales of crossing a curved barrier with that of the memory associated with the non-Markovian dynamics

2.4.4 Water permeability

In the previous section, we demonstrated by means of BD_e simulations the effectiveness of the committor time-correlation function for determining the exact permeability for two simple model systems, even when the ISD model fails. We now apply this framework to the treatment of water permeation across a membrane. In order to validate the committor time-correlation function for determining permeability from MD simulations, we choose wa-

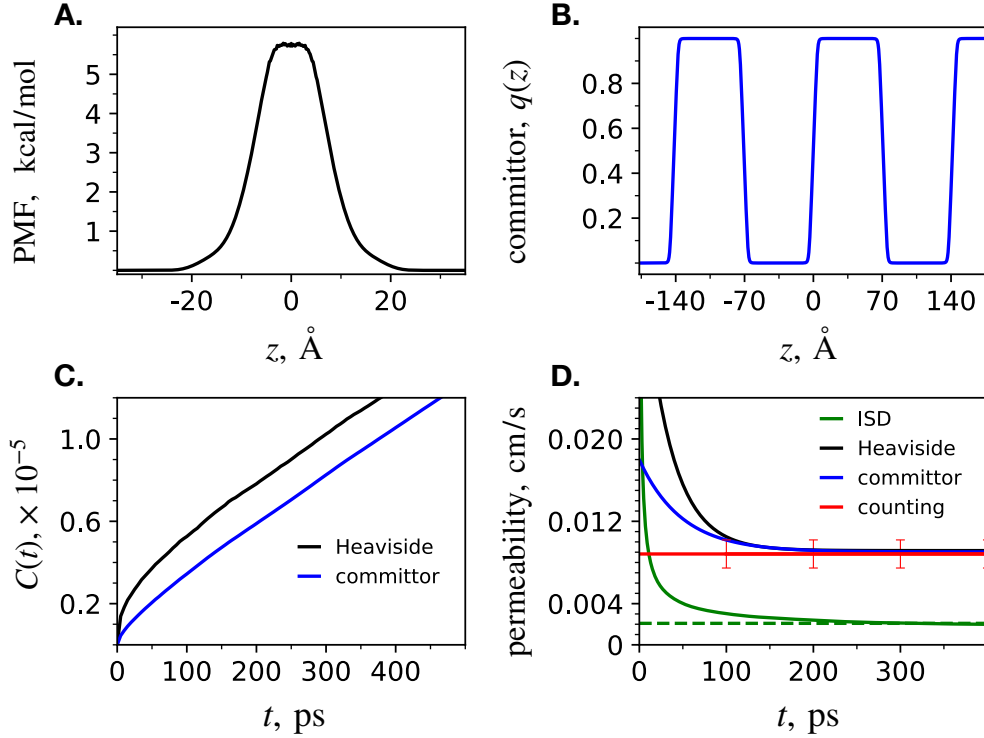


Figure 2.9: **A.** PMF of water across a DLPC bilayer, computed from the marginal probability distribution from unbiased MD with the Drude2023 force field.³⁰ **B.** Approximate committor of water, which is “unwrapped”, or extended, to cover several periodic images of the membrane system. The committor is calculated from the PMF assuming that the diffusivity is independent of z . **C.** Committor ($C_{qq}(t)$) and Heaviside ($C_{ab}(t)$) time-correlation functions, averaged from each unwrapped water trajectory, mapped onto the unwrapped committor and Heaviside function, respectively. **D.** Permeability of DLPC to water based on the ISD model (green) with eq 2.1, “Heaviside” (black) with eq 2.9, “committor” (blue) with eq 2.19, and “counting” (red) with eq 2.13. The ISD permeability depends on the position-dependent diffusivity $D(z)$, which was determined with biased US simulations. The lag time dependence of the ISD is related to the upper limit of the integral of the position autocorrelation function (see section S4 of the SI).⁵ The dashed green line corresponds to an ISD estimate using the $D(z)$ profile of Figure S4. The counting permeability is based on a mean and standard deviation of 52.0 ± 8.0 crossings per replicate, where three replicates were simulated for 400 ns each.

ter permeation because unbiased simulations on reasonable timescales will be sufficient for counting crossing events, computing the PMF based on the marginal probability distribution of water along z , and computing the committor time-correlation function.

The Drude2023 force field³⁰ was used to represent a DLPC lipid bilayer with SWM4 water.⁸⁰ The PMF of water along the z -axis, necessary for approximating the committor, is shown in Figure 2.9A. In Figure 2.9B., the “unwrapped” approximate committor is shown. It was constructed based on eq 2.20, and extended to cover several periodic images. The committor time-correlation function in Figure 2.9C. was then computed by mapping the “unwrapped” trajectories of each water z onto the “unwrapped” committor. Although there is a large number of water molecules in the system, the probability of finding one such molecule in the membrane is small. Thus, the cross-terms between different water molecules can be neglected in the committor time-correlation function, and the final expression only involves an average over a single permeant molecule.

A comparison of the water permeability computed by the ISD model (eq 2.1), Heaviside time-correlation function (eq 2.9), committor time-correlation function (eq 2.19), and counting crossing events (eq 2.13) is shown in Figure 2.9D. We note that the ISD permeability estimate required US simulations, because the MSD method for computing diffusivity is not reliable for unbiased simulations in cases where the PMF changes quickly along z (see Figure 2.4). Analysis of the US position-dependent diffusivity is included in section S4 of the SI.

As was observed for the BD_e systems, the committor and counting permeability agree very closely after a lag time on the order of hundreds of picoseconds. Furthermore, the ISD permeability plateau value for water underestimates the exact permeability. In particular, the counting permeability was found to be 0.00883 cm/s, very close to the committor plateau (400 ps) permeability of 0.00903 cm/s. The ISD permeability at the plateau is just 0.00209 cm/s, indicating a relatively slowly decaying memory function—or small K_e compared to

W'' —within the framework of an exponential memory. Here, we can estimate the ratio of τ_e over τ_0 for water permeation of the DLPC membrane based on eq A.55. The fraction of the exact permeability over the ISD permeability is about 4.32, which corresponds to the magnitude of the factor $1/(1 - \tau_e/\tau_0)$. The ratio τ_e/τ_0 is therefore 0.769.

The time needed by a water molecule to successfully cross the membrane during a permeation event, i.e., the mean reactive transit time $\langle\tau_{\text{r}}\rangle$, can be determined using the committor-based expression,²⁶

$$\langle\tau_{\text{r}}\rangle = \frac{1}{P} \int_{z_a}^{z_b} dz q(z) (1 - q(z)) e^{-[W(z) - W(z_a)]/k_{\text{B}}T} \quad (2.37)$$

where $z_a = -L/2$ and $z_b = L/2$. If P is set to the calculated permeability (Figure 2.9), and $\pm L/2$ is set to ± 30 Å, the calculated mean reactive transit time is 2.44 ns. The actual mean reactive transit time calculated directly from the MD trajectory of each water is 2.93 ns. The successful crossing events from the “unwrapped” water trajectory are shown in Figure 2.10. The good agreement between the calculated mean transit time with the committor expression and the value extracted from the trajectory is evidence that the approximate committor provides a meaningful framework for modeling water permeation.

2.5 Concluding Discussion

The nature of the permeation process can vary markedly depending on the atomic details of the system. This has critical consequences on the choice of an effective computational strategy. When the free-energy barrier to permeation is low, the permeability coefficient can be determined directly by counting the number of spontaneous, uncorrelated crossing events over the course of a standard unbiased equilibrium MD trajectory.^{12;1;51;52} However, when the free-energy barrier is large, it becomes increasingly unlikely that spontaneous membrane crossing events can be observed during an unbiased trajectory, and one must

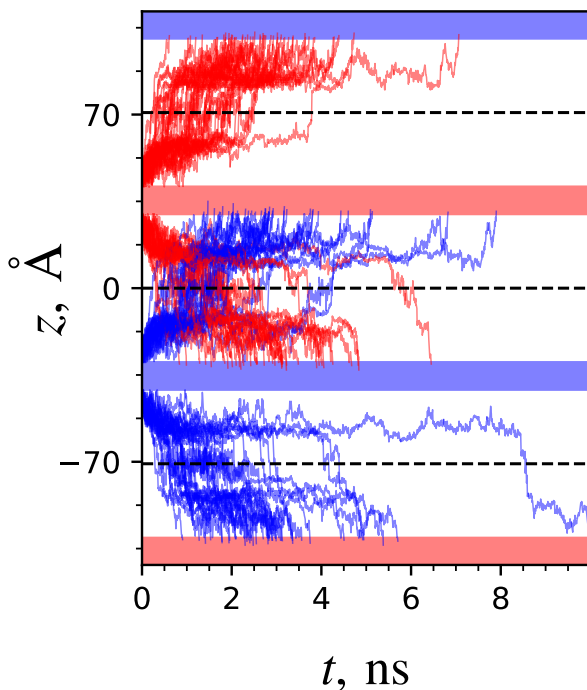


Figure 2.10: Successful water crossings beginning at the time the molecule left region *a* (red) or *b* (blue) and ending at the time at which it first touches the opposite region. The trajectories are colored based on the initial region. The dashed black line is the center of each image of the membrane (see Figure 2.9B.). The mean reactive transit time is interpreted as the mean duration of the crossings, which is 2.93 ns.

utilize some enhanced sampling methodology to determine the membrane permeability to a given molecule. In this context, the ISD (inhomogeneous solubility-diffusion) model based on eq 2.1 is a widely used approximate framework to determine the permeability coefficient.^{6;35;36;37;38;39;40}

The two principal ingredients of the ISD model are the PMF, $W(z)$, of the permeant molecule along the z -axis, parallel to the membrane normal, and the position-dependent diffusivity profile, $D(z)$. The PMF is a rigorously defined equilibrium quantity,⁴¹ and several rigorous enhanced sampling strategies are available for its calculation.^{42;43;10;44;45;46} However, the diffusivity profile is a dynamical transport coefficient, which may be difficult to estimate in the context of small molecules in membranes, which often exhibit slowly decaying memory effects.⁴⁹ One standard approach to determine $D(z)$ consists in

considering the mean-squared displacement (MSD) for a lag time t , using the relation, $2Dt \sim \langle \Delta(z(t) - z(0))^2 \rangle$, which is valid in the diffusive limit. Estimates of D based on the MSD may display a strong dependence on the lag time t , which may be indicative of fundamental issues with the diffusive limit. In a uniform liquid, the dynamics is affected by non-Markovian memory effects, and an estimate of D based on the MSD is expected to yield an accurate effective diffusivity, D_{eff} , only when the lag time t is sufficiently long to achieve the diffusive limit. Thus, the MSD correctly converges to the diffusive limit in a homogeneous system with a flat free-energy profile, or with a flattened profile as in ABF or metadynamics simulations.^{44;45} However, when there is a well or a barrier, the estimate of D based on the MSD is shown to rapidly deviate from any reasonable plateau.

It is imperative to formulate alternative theoretical and computational routes for the determination of the permeability coefficient allowing us to go beyond the limited ISD model. For this purpose, we returned to Green-Kubo statistical mechanical linear response theory. A general expression of the permeability coefficient was formulated in terms of an equilibrium time-correlation function, recovering the constitutive relation of membrane permeation, $j = P\Delta C$. The main formal results from our analysis are embodied in eq 2.9 and eq 2.10. Moreover, inspired by the counting method (eq 2.13), an additional result expressing the permeability coefficient in terms of the committor time-correlation function (eq 2.19) was obtained using TPT. These formal expressions provide useful starting points to design novel enhanced sampling strategies for the determination of permeability coefficients. The analysis does not make any assumption about the nature of the microscopic dynamics of the permeation process.

The validity of eq 2.9, eq 2.10, and eq 2.19 was tested using non-Markovian BD simulations with memory for two simple barrier models. The results from these simulations show that the different statistical mechanical formulations are consistent with the simple counting method. Some interesting lessons could be drawn from the non-Markovian BD

simulations, illustrating the importance of various factors associated with the memory function and barrier curvature that influence membrane permeability. In particular, we showed how the timescale of the memory function can overlap with the timescale of crossing a given barrier. Essentially, the total friction may not be felt by the permeant molecule as it crosses the free-energy barrier. This observation implies that, when properly accounted for, memory effects yield a smaller friction and a larger permeability coefficient than that estimated from the ISD approximation, which must assume the total friction. Ultimately, these observations point to the main issue at the heart of the ISD approximation, which is the underlying assumption of diffusive dynamics. This assumption is, at best, of unknown validity in most cases.

As a last example, the permeation of water molecules across a lipid membrane using all-atom MD simulation based on the Drude polarizable force field was examined to illustrate the new statistical mechanical theories developed here. Numerous spontaneous crossing transitions of the membrane allow a direct determination of the permeability coefficient. Again, the results showed that the different statistical mechanical formulations are consistent with the simple counting method.

We are particularly interested in further exploring the utility of the committor time-correlation function $C_{qq}(t)$ (eq 2.19) to determine the permeation coefficient. Such a time-correlation function can be rigorously calculated from short unbiased trajectories with enhanced sampling strategies using the re-weighting procedure based on eq 2.36. In future work, we shall use this approach to characterize the permeation of molecules with high free-energy barriers.

CHAPTER 3

MEMBRANE PERMEABILITY OF SUCROSE CALCULATED FROM EQUILIBRIUM TIME-CORRELATION FUNCTIONS USING MOLECULAR DYNAMICS SIMULATIONS WITH ENHANCED SAMPLING

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<https://pubs.acs.org/doi/full/10.1021/acs.jpcb.5c02905>

ABSTRACT – A computational framework for rigorously computing the membrane permeability of small molecules from unbiased molecular dynamics simulations is presented. The method in its optimized form, which exploits the committor probability within the transition path theory framework, is applicable to molecules with free energy barriers for which a determination of the permeability coefficient from spontaneous crossings during equilibrium simulations would be unfeasible. A novel computational protocol is implemented through which the equilibrium time-correlation function is calculated from a combination of enhanced sampling to determine the equilibrium potential of mean force of the permeant molecule and a re-weighted ensemble of short unbiased trajectories. By extracting the steady-state flux from committor-based equilibrium time-correlation functions of unbiased dynamics, the method provides a rigorous computational route to determine the permeability coefficient while going beyond the inhomogeneous solubility diffusion (ISD) model, which is built upon the assumption of overdamped dynamics. The highly polar, slowly permeating molecule

sucrose is used here as an illustrative example of the committor-based permeability theory. For a full assessment of how the method compares to the ISD model, systems of DOPC and DLPC membranes are considered for both additive and polarizable force field models. Estimates based on the ISD permeability are systematically smaller than or equal to the values determined from the present method, indicating that non-Markovian memory effects that do not fully decay on the timescale of membrane crossing are potentially ignored.

3.1 Introduction

Calculating the membrane permeability of small molecules from all-atom molecular dynamics (MD) simulations can be a challenging problem because the slow permeation process may require prohibitively long trajectories. It is possible, for example, to determine reliably the permeability coefficient of water from unbiased trajectories by counting spontaneous crossings,^{50;12;1;51;52} or that of some small molecules via transition path sampling (TPS).^{53;86;87}

In one application of the replica exchange transition interface sampling (RETIS) method, the permeability of oxygen from MD simulations was shown to be in agreement with corresponding unbiased counting results.⁸⁸ This method has not as of yet been applied to MD simulations of small molecules with significant barriers to permeation, however. Further, it is generally not possible to rely solely on unbiased MD simulations¹¹ for molecules that permeate slowly, and it is necessary to use an enhanced sampling strategy in conjunction with some level of theory for extracting the permeability coefficient.⁴⁰ For this reason, developing alternative theoretical and computational frameworks that allow an exact calculation of membrane permeability from reasonable computational resources is critically important.

The most widely used approach is perhaps the inhomogeneous solubility-diffusion (ISD) approximation,⁸ which requires the calculation of the potential of mean force (PMF) and position-dependent diffusivity of the permeant molecule along the axis perpendicular to the membrane.^{38;26} However, the ISD approximation may or may not yield a reasonable esti-

mate of the permeability coefficient from molecule to molecule and membrane to membrane. The central issue is that it is an approximation based on the assumption that the permeant molecule is crossing the membrane as an overdamped diffusion, which is genuinely of unknown validity in most cases.⁸ Alternatively, Markov state models (MSM),^{54;55} Milestoning,^{56;57;58;59;60} and weighted ensemble (WE)⁶¹ are three approaches to compute the exact permeability without relying on the assumptions inherent in the ISD model.

In previous work, we formulated a statistical mechanical theory of membrane permeation within the frameworks of Green-Kubo linear response theory and the committor probability, of which the latter is taken in the context of transition path theory (TPT).¹³ The permeability coefficient could be rigorously expressed in terms of different equilibrium time-correlation functions.¹³ The approach was illustrated and validated with the permeation of water molecules by relying on unbiased trajectories to calculate the time-correlation functions. However, the feasibility of the equilibrium simulation method for calculating membrane permeability does not extend very far beyond water. The advantages of a formulation based on equilibrium time-correlation functions,¹³ from a practical standpoint, is that the latter can be evaluated rigorously using enhanced sampling strategies. Importantly, the linear response formulation of permeability is straightforwardly rigorous and makes no assumptions about the nature of the dynamics of the permeant molecules, in contrast to treatments based on ISD,⁶ MSM,^{54;55} Milestoning,^{56;57;58;59;60} and WE.⁶¹

Our objective here is to assess the ability of our theoretical formulation to treat a slowly permeating molecule using MD simulations with enhanced sampling strategies. To illustrate the approach, we consider the highly polar, slowly permeating molecule sucrose. Aside from its impact on health, sucrose has been investigated for its function as a cryoprotectant,⁸⁹ permeability marker of the blood-brain barrier (BBB),^{90;91} and inert osmolyte in vesicle experiments to induce a unidirectional net flux.^{92;29;93} While chemically sucrose is a medium-sized, highly polar molecule that is likely to have a small membrane permeabil-

ity, experimental and computational work on accurately determining this value is almost nonexistent. The permeation of sucrose is expected to be a rare event that cannot be readily observed with unbiased simulations.^{94;95} In order to characterize the permeability of sucrose in a comprehensive way, we consider two membrane compositions, two force fields, and two levels of theory. Specifically, we consider saturated (DLPC) and unsaturated (DOPC) membranes, the CHARMM36 (C36) additive and Drude2023 polarizable force fields, and the levels of theory provided by the standard ISD approximation and the new committor-based method.

This effort to compare the C36 and Drude force fields for a permeation process is preceded by the work of others. Allen and coworkers⁹⁶ characterized two force field and membrane thickness dependent mechanisms of the permeation of a charged small molecule. They found that for the additive force field and thinner membranes, the charged molecule permeates with an ion defect mechanism. However, if the membrane is thick enough or the polarizable force field is used, the molecule can be treated with the standard theory of permeation. In addition, Lemkul and coworkers⁹⁷ have shown that the barrier across a membrane is substantially different depending on the polarizability of the force field for a range of neutral and charged amino acid side-chains.

In the next sections, we first review the expressions for computing the permeability coefficient based on the committor, indicator, and indicator-restricted committor that we derived previously.¹³ We then introduce the new contribution of the practical implementation of these methods for MD simulations with PMFs that would be unfeasible to compute by brute force and must rely on enhanced sampling. Next, we outline the simulations and protocol used in this study for computing the permeability of DOPC and DLPC membranes to sucrose with additive and polarizable force fields. The MD results consist of the PMFs and position-dependent diffusivity profiles, as well as the permeability values computed with each time-correlation function and with the ISD model. We also provide a numerical validation of

the time-correlation expressions of the permeability and the re-weighting method of short unbiased trajectories by integrating stochastic simulations of 2-dimensional Brownian dynamics with non-Markovian memory effects. Overall, we present a practical approach for computing exact permeability coefficients from enhanced sampling simulations, while characterizing the permeation of sucrose under different simulation conditions as an example.

3.2 Theoretical developments

In addition to the classic inhomogeneous solubility-diffusion (ISD),^{38;26} the main theoretical treatments of membrane permeation considered in the present study involve time-correlation functions from Green-Kubo linear response and transition path theory (TPT). These treatments, which were introduced previously,¹³ are briefly summarized in the following for the sake of completeness.

3.2.1 Inhomogeneous solubility-diffusion (ISD)

A popular approximation to compute membrane permeability from enhanced sampling MD^{38;26} is the ISD approximation,⁸

$$P = \left[\int_{-L/2}^{L/2} dz \frac{e^{[W(z)-W(-L/2)]/k_B T}}{D(z)} \right]^{-1} \quad (3.1)$$

where $W(z)$ is the potential of mean force (PMF) and $D(z)$ is the position-dependent diffusivity. The width of the membrane is L , where z is aligned with the membrane normal. The center of the membrane is located at $z = 0$, and the PMF is assumed to reach a constant value where $|z| > L/2$, which can be set to zero for convenience. This expression assumes that the dynamics of the molecule within the membrane, when projected onto the z -axis, fall within the overdamped regime. The position-dependent diffusivity must be estimated using one of several methods, depending on the enhanced sampling method that has been

deployed, such as the methods of Crouzy et al.⁹⁸ or Hummer⁵ for umbrella sampling, or Im and Roux⁹⁹ for adaptive biasing force. Within the ISD framework, the memory function of the molecule in the membrane is assumed to decay quickly, such that the position-dependent diffusivity corresponds to a valid estimate of the molecule’s diffusivity during a genuine membrane crossing event. In some cases, however, it is expected that high curvature near the top of the PMF and a relatively slowly decaying memory function with respect to z invalidate the assumptions of the ISD model.

3.2.2 *Time-correlation formulation of membrane permeability*

We have shown previously that the exact permeability coefficient can be extracted from the limiting slope of various time-correlation functions.¹³ One expression for the permeability derived from Green-Kubo linear response theory involves indicator functions $H_a(z)$ and $H_b(z)$ which are 1 and 0 respectively when $z < 0$, and vice versa when $z > 0$. The exact permeability is,

$$P = \frac{1}{\pi(-L/2)} \lim_{t \rightarrow \tau_H} \dot{C}_{ab}(t) \quad (3.2)$$

where $\pi(-L/2)$ is the equilibrium probability in solution near the edge of the membrane. The indicator time-correlation is,¹³

$$C_{ab}(t) = \langle H_a(0)H_b(t) \rangle \quad (3.3)$$

and τ_H is the timescale at which the slope of the Heaviside time-correlation function reaches a plateau. An alternative expression derived from TPT involves a time-correlation function of the committor probability $q(z)$,

$$P = \frac{1}{\pi(-L/2)} \lim_{t \rightarrow \tau_q} \dot{C}_{qq}(t) \quad (3.4)$$

where the committor time-correlation function is,

$$C_{qq}(t) = \frac{1}{2} \langle (q(t) - q(0))^2 \rangle \quad (3.5)$$

and τ_q is another finite relaxation time. It may be noted that τ_q is typically shorter than τ_H . Lastly, the permeability may also be expressed from the limiting slope of an indicator-restricted committor time-correlation function,¹⁸

$$P = \frac{1}{\pi(-L/2)} \lim_{t \rightarrow \tau_{H-q}} \dot{C}_{ab-qq} \quad (3.6)$$

where the time-correlation function is given by,

$$C_{ab-qq}(t) = \langle H_a(0) H_b(t) (q(t) - q(0)) \rangle \quad (3.7)$$

where τ_{H-q} is a relaxation time expected to fall between τ_H and τ_q described above. These different time-correlation functions are expected to reach to a limiting slope with different relaxation times. As discussed in previous work,^{13;18} these relaxation times, τ_H , τ_q , and τ_{H-q} are related to the decay rate of orthogonal modes in a Markovian spectral decomposition of the different time-correlation functions.

In eq 3.5 we have used a simplified notation for clarity, such that $q(t)$ represents $q(z(t))$, the value of z at time t mapped onto the committor probability. An analogous simplification was made in eq 3.3 and eq 3.7 for each indicator function. While each of these expressions will theoretically lead to the same value of the permeability, they are not necessarily expected to converge over the same timescale. The lag time needed to reach the plateau value of the derivative of each time-correlation function must be independently examined.

Strictly speaking, the committor $q(z)$, corresponding to the probability that the permeant will cross to the opposite side of the membrane before returning to the original side, given

its current position at z , must be determined either by short unbiased trajectories or from the variational principle. However, under the approximation of overdamped dynamics in one dimension, a simple form can be written,¹³

$$q(z) = \frac{\int_{-L/2}^z dz' e^{W(z')/k_B T} / D(z')}{\int_{-L/2}^{L/2} dz' e^{W(z')/k_B T} / D(z')} \quad (3.8)$$

In practice, it is assumed that the diffusivity is constant with respect to z for the sake of simplicity. While eq 3.8 is an approximation of the exact committor, it can still be used in the time-correlation functions above to extract the exact permeability with a proper lag time.¹⁸ It can be shown that, while the exact committor is closely related to the eigenvector of the effective dynamical propagator with the slowest relaxation, the overlaps of an approximate committor with higher order eigenvectors decays to zero within a finite lag time.¹⁸

3.2.3 *Evaluation of the time-correlation functions*

The equilibrium time-correlation functions given by eq 3.3, eq 3.5, and eq 3.7 are central quantities for determining the permeability coefficient. In the simplest application, the time-correlation functions described in the previous section are computed from an equilibrium ensemble of trajectories which have sufficiently sampled the entire z -coordinate across the membrane system. However, a molecule like sucrose would not spontaneously cross the membrane interior on a practical timescale. In such a case, it is necessary to compute the PMF with an enhanced-sampling method first, and then to generate short unbiased trajectories along z . We demonstrated previously with Brownian dynamics simulations that it is possible to compute the committor time-correlation function from a set of short unbiased trajectories with initial positions taken from a uniform random distribution through re-weighting with the known equilibrium probabilities.^{13;100} Sampling of time-correlation functions involving

slow processes using short unbiased trajectories requires a careful staging of initial conditions of the permeant molecule along the z -axis in order to overcome the challenge posed by the free energy barrier at the center of the membrane. In this regard, it is of interest to seek an optimal sampling strategy in which we make the best use of the short unbiased MD trajectories along the z -axis. Let us first express the time-correlation function of interest as a sum of the initial conditions over N bins along z , where z_n refers to each discrete initial position,

$$\begin{aligned} C(t^*) &= \int dz \pi(z) c(t^*; z) \\ &= \sum_{n=1}^N p(z_n) c(t^*; z_n) \end{aligned} \quad (3.9)$$

where $p(z_n) = \pi(z_n)\Delta z$, and $c(t^*; z_n)$ is a conditional time-correlation function, or the value of the time-correlation of interest given the initial position z_n . The conditional time-correlation is an average over time,

$$c(t^*; z_n) = \frac{1}{n_t} \sum_{k=1}^{n_t} F(k\Delta t, k\Delta t + t^*; z_n) \quad (3.10)$$

where $t = k\Delta t$ represents the time step of the trajectories, and $F(k\Delta t, k\Delta t + t^*; z_n)$ is the particular time-correlation function of interest. This expression is a standard average for a time-correlation function obtained by moving the origin of time. It is valid here because the different contributions are weighted by the equilibrium distribution $\pi(z)$, which ensures that there is no origin of time. Each of the three time-correlation functions introduced above, the committor (eq 3.5), indicator (eq 3.3), and indicator-restricted committor (eq 3.7), can be re-weighted with eq 3.9. We choose the appropriate form of $F(t, t + t^*)$, which is the mathematical expression corresponding to the time-correlation function of interest, defined explicitly for each case in the following equations, to insert into eq 3.10. Specifically, for the

committor time-correlation function,

$$F(t, t + t^*) = \frac{1}{2} (q(t + t^*) - q(t))^2 \quad (3.11)$$

indicator time-correlation function,

$$F(t + t^*; t) = \frac{1}{2} (H_a(t)H_b(t + t^*) + H_a(t + t^*)H_b(t)) \quad (3.12)$$

and indicator-restricted time-correlation function,

$$\begin{aligned} F(t, t + t^*) &= \frac{1}{2} (H_a(t)H_b(t + t^*)(q(t + t^*) - q(t)) \\ &\quad + H_a(t + t^*)H_b(t)(q(t) - q(t + t^*))) \end{aligned} \quad (3.13)$$

In the first case, the symmetry of the membrane permeation process is built-in to the committor time-correlation function with the quadratic term. In the last two cases, we impose symmetry over the center of the membrane.

To use these expressions, many unbiased trajectories with initial positions that sample the entire z -axis, each of duration at least as long as the relaxation time of the slowest relaxing time-correlation function, are required. There are many ways to decrease the statistical uncertainty on the calculated time-correlation function. For instance, one could simply increase the number of simulations initiated in each z_n bin. However, it is not immediately clear which z_n initial positions are most important to sample for convergence. It is apparent that both the equilibrium probability, $\pi(z_n)$, and the local magnitude of the conditional time-correlation function itself, $c(t^*; z_n)$, will affect the convergence of the calculation and its statistical uncertainty. To systematically assess the uncertainty in the re-weighted time-correlation functions, we provide a scheme for calculating the variance in each z_n bin. The

estimator of the mean time-correlation function (eq 3.6) is,

$$\overline{C(t^*)} = \sum_{n=1}^N p(z_n) \frac{1}{w_n} \sum_{i_n=1}^{w_n} c_{i_n}(t^*; z_n) \quad (3.14)$$

where $c_{i_n}(t^*; z_n)$ is the outcome from simulation i_n which is initiated in bin z_n , and the weights $(w_1, \dots, w_N)$ are the total number of simulations initiated in each bin. It is easy to show that the expectation value of the estimator, $E\{\overline{C(t^*)}\}$, provides the desired correlation function $C(t^*)$. We now consider the mathematical expectation value for the variance of the estimator. Defining the deviations from the expected value as $\delta\overline{C(t^*)} = \overline{C(t^*)} - E\{\overline{C(t^*)}\}$, $\delta c_{i_n}(t^*; z_n) = c_{i_n}(t^*; z_n) - E\{c_{i_n}(t^*; z_n)\}$, we write,

$$\begin{aligned} E\left\{\left(\delta\overline{C(t^*)}\right)^2\right\} &= E\left\{\left(\frac{1}{w_n} \sum_{i_n=1}^{w_n} \sum_{n=1}^N p(z_n) \delta c_{i_n}(t^*; z_n)\right)^2\right\} \\ &= E\left\{\left(\frac{1}{w_n} \sum_{i_n=1}^{w_n} \sum_{n=1}^N p(z_n) \delta c_{i_n}(t^*; z_n)\right) \right. \\ &\quad \left. \left(\frac{1}{w_m} \sum_{j_m=1}^{w_m} \sum_{m=1}^N p(z_m) \delta c_{j_m}(t^*; z_m)\right)\right\} \\ &= \sum_{i_n=1}^{w_n} \sum_{j_m=1}^{w_m} \sum_{n=1}^N \sum_{m=1}^N \frac{p(z_n)}{w_n} \frac{p(z_m)}{w_m} E\left\{\delta c_{i_n}(t^*; z_n) \delta c_{j_m}(t^*; z_m)\right\} \\ &= \sum_{i_n=1}^{w_n} \sum_{j_m=1}^{w_m} \sum_{n=1}^N \sum_{m=1}^N \frac{p(z_n)}{w_n} \frac{p(z_m)}{w_m} \sigma_{z_n, i_n, z_m, j_m}^2 \end{aligned} \quad (3.15)$$

In the present calculation, different trajectories were generated independently from different initial conditions. Therefore, there is no reason to expect that different trajectories launched from different initial conditions either in the same or different bins are statistically correlated.

For this reason, it is reasonable to assume that $\sigma_{z_n, i_n, z_m, j_m}^2 = \delta_{n,m} \delta_{i_n, j_m} \sigma_{z_n}^2$, which yields,

$$\sigma_{\text{tot}}^2 = \sum_{n=1}^N \frac{p(z_n)^2}{w_n} \sigma_{z_n}^2 \quad (3.16)$$

Here, $\sigma_{z_n}^2$ is the population variance calculated from the set of independent simulations,

$$\sigma_{z_n}^2 = \frac{1}{w_n} \sum_{i_n=1}^{w_n} \left(c_{i_n}(t^*; z_n) \right)^2 - \left(\frac{1}{w_n} \sum_{i_n=1}^{w_n} c_{i_n}(t^*; z_n) \right)^2 \quad (3.17)$$

Based on eq 3.16, it is clear that increasing the number of simulations, w_n , for the bins z_n that have the largest amplitude, $p(z_n)^2 \sigma_{z_n}^2$, will be the most efficient way to minimize the global variance of our estimator of the time-correlation function $\overline{C(t^*)}$ as defined in eq 3.14.

3.3 Simulations and Methods

3.3.1 Numerical validation from stochastic simulations

Stochastic simulations of a simple model system were used to validate the expressions of permeability based on the re-weighted time-correlation functions. To generate stochastic trajectories with non-Markovian memory effects, we used the method described in our previous work for constructing dynamics with an effective exponential memory function, whereby a set of 2-dimensional Brownian dynamics (BD) simulations are integrated and then projected onto the z collective variable.¹³ The coupled equations governing the position of the Brownian particle in each dimension are given by,

$$z(t + \Delta t) = z(t) - \frac{D_z}{k_B T} \left(\frac{\partial W(z)}{\partial z} + K_e(z - z_e) \right) + g_z \quad (3.18)$$

$$z_e(t + \Delta t) = z_e(t) + \frac{D_e}{k_B T} K_e(z - z_e) \Delta t + g_e \quad (3.19)$$

where z is the coordinate that is perpendicular to the membrane and z_e is the auxiliary variable. The other parameters include $W(z)$, which is the PMF, D_z , the diffusivity with respect to z , D_e , the diffusivity with respect to z_e , and K_e , the harmonic force constant. The Gaussian noise terms for z and z_e are denoted by g_z and g_e . They have mean values of zero, and variances of $2D_z\Delta t$ and $2D_e\Delta t$. When constructed in this way, dynamics along z will exhibit a memory function within the framework of the overdamped generalized Langevin equation (GLE).¹⁴ The instantaneous diffusivity along z is simply D_z , and the effective diffusivity after the decay of the memory function is $1/(1/D_z + 1/D_e)$.

The dimensional values chosen for the BD simulations are as follows. The PMF is a Gaussian function scaled by a factor of 4 kcal/mol, such that $W(z) = 4e^{-z^2/125}$ kcal/mol. The diffusivity is given by, $D_z = D_e = 0.1 \text{ \AA}^2/\text{ps}$, and the harmonic coupling constant is, $K_e = 0.05 \text{ kcal/mol \AA}^2$. All of the simulations were run with a 0.01 ps timestep. The temperature was set to 308 K. Boundaries were set by reflecting hard walls where $z = \pm 100 \text{ \AA}$. No boundaries were applied for z_e .

Table 3.1: Summary of 2D-Brownian dynamics simulations

description	replicates	time/rep	total time (μs)
equilibrium	593	50 μs	29,650
short trajectories	4×10^6	500 ps	2000

The sets of simulations used for evaluating the permeability of the system are summarized in Table 3.1. First, brute force simulations were run for computing the counting permeability¹ and each correlation function from an equilibrium ensemble. A summary of the observed numbers of membrane crossing events is provided in Figure B.2 in Appendix B. Then, short trajectories were run for validating the method for re-weighting the correlation functions that is presented here. For all of the short trajectories, the initial z_e (auxiliary variable) positions were taken from its equilibrium distribution, which is that of a harmonic potential with a force constant of K_e . Properly equilibrating the z_e positions is important

for reproducing the counting and equilibrium time-correlation permeability.

As an illustration of the effectiveness of minimizing the global variance given by eq 3.16 within each z_n bin for obtaining well-converged time-correlation functions, we present an automatic method for determining the optimal distribution of initial positions. While a general closed-form expression predicting the optimal distribution of initial positions that will minimize the global variance with the least amount of sampling would be desirable, we are not aware of such a result. The difficulty arises from the different competing factors of the equilibrium probability, $\pi(z)$, and the magnitude of the conditional time-correlation function, $c(t^*; z_n)$ with respect to z_n . While we suspect that the latter will be proportional to the square of the derivative of the committor probability, this behavior is counteracted by the contribution of $\pi(z)$. Notably, the predicted maximum of $c(t^*; z_n)$ occurs at the center of the membrane, where $\pi(z)$ reaches its minimum.

The scheme for iteratively optimizing the distribution of the short unbiased simulations proceeded as follows. For 20 iterations each consisting of 4000 trajectories, the change in variance in each bin is computed and used to construct the initial distribution for the next iteration. The initial distribution of the first iteration is simply uniform. We begin with a uniform distribution to demonstrate that the scheme will lead towards an optimized distribution even if we have no intuition about what it might be for our system. For the purpose of the optimization of sampling for the 2-dimensional BD simulations, the change in variance is defined as,

$$\Delta\sigma_{z_n}^2 = p(z_n)^2 \frac{|\sigma_{z_n}^2(w_n) - \sigma_{z_n}^2(w_n/2)|}{w_n/2} \quad (3.20)$$

where $\sigma_{z_n}^2(w_n)$ is the population variance of bin z_n as a function of the number of trajectories w_n with this initial position, given explicitly by eq 3.17. We chose the committor time-correlation function (eq 3.11) as the basis of the optimization. The lag time t^* was chosen to be 300 ps. The resulting distribution over z_n is then normalized, symmetrized about

$z = 0$, and smoothed with a rolling average with a 2 Å window. The 4000 trajectories for the next iteration are then initiated by generating 4000 uniform random numbers from 0 to 1 and converting them to z_n values through the inverse cumulative distribution function (CDF), $P^{-1}(\Delta\sigma_{z_n}^2)$. For computational efficiency, 50 parallel sets of 20 serial iterations were run, each beginning from an independent uniform distribution. The total number of short trajectories recorded in Table 3.1, therefore, is 50 sets times 20 iterations times 4000 trajectories per iteration. An illustration of the evolution of the standard error compared to that of strictly uniform sampling and the cumulative distribution function obtained for each iteration are shown in Figure B.3 and Figure B.4, respectively, in Appendix B.

3.3.2 *Optimal sampling with short trajectories and variance reduction*

In order to compute the exact permeability coefficients of each membrane system from equilibrium time-correlation functions, we first computed the PMF of sucrose across each force field and membrane system with umbrella sampling (US) simulations. In summary, the US simulations consisted of one window per Å, from $z = -36$ to $z = +36$ Å. A second window centered at $z = 0$ was also simulated. More details about the US simulations are provided in section c) of Simulations and Methods.

Next, a large number of short unbiased trajectories were generated for each system. Each set of trajectories contained 45 initial configurations for every 1 Å along z from -42 to $+42$ Å for the C36 systems and -40 to $+40$ Å for the Drude systems. The initial configurations were saved from the restrained US trajectory of each window from $z = -36$ to $+36$, with frames saved every 1 ns for 45 ns, starting after the first 5 ns of simulation time. For $z = 0$, an additional 45 starting points were saved from the second umbrella window at $z = 0$. For $z > +36$ and $z < -36$ Å, the starting points were saved from an unbiased trajectory in the bulk. To generate each short trajectory, the original umbrella restraints were then turned on for a brief 100 ps equilibration, and then released for 400 ps of unbiased simulation.

Each of the initial configurations was simulated for two independent trajectories. To clarify, for each independent trajectory with the same initial configuration, the initial coordinates before equilibration were identical, however the velocities were randomized via a new seed and 100 ps equilibration.

As an initial guess of the optimal distribution to minimize the uncertainty in the mean time-correlation functions, $z = 0$ was chosen for extra sampling. The 90 initial configurations (45 from each umbrella window at $z = 0$) were generated and simulated for 8 additional independent trajectories each for the C36 systems and 18 additional independent trajectories each for the Drude systems. Finally, based on the assessment of the convergence of the time-correlation functions for each system, it was determined that additional sampling was needed for both of the DLPC membrane systems. Therefore, 8 additional independent trajectories were generated from each of the 45 initial configuration located at $z = -3, -2, -1, 1, 2, 3$ Å for both of the DLPC systems. More details on the error assessment of the short unbiased MD simulations are given in Appendix B. In total, there were 8460 trajectories for C36 DOPC, 10620 for C36 DLPC, 9000 for Drude DOPC, and 11160 for Drude DLPC. In terms of simulation time, there were 3.384 μ s for C36 DOPC 4.248 μ s for C36 DLPC, and 3.6 μ s Drude DOPC and 4.464 μ s for Drude DLPC.

In practice, each time-correlation function was calculated from the time series of the z collective variable evaluated without accounting for periodic images. It was therefore necessary to unwrap the committor and indicator functions before mapping the z coordinates to the relevant function. This unwrapping was accomplished using the average z -dimension of each of the simulation boxes from the set of short trajectories (which were run with NPT). This approximation is not expected to have any significant impact on the resulting time-correlation functions because (1) the trajectories are too short to allow for large sojourns into periodic images and (2) the slope of committor is small far from the center of the membrane. The resulting committor and indicator functions for each membrane system are

provided in Figure B.5 in Appendix B. The time-correlation functions were then properly re-weighted to the equilibrium ensemble via eq 3.9, which required the PMF calculated with US. The analytical derivative of each time-correlation function was extracted by fitting the differentiable function, $C(t) \approx -ae^{-st} + mt + d$.

The errorbars for each time-correlation function are plus and minus the standard error, which was calculated as the square root of the global variance given by eq 3.16. In addition, we considered the relative error in each z_n bin for the committor time-correlation function of each system, evaluated at a lag time $t^* = 300$ ps. We define the relative error as the standard error in a single bin, divided by the magnitude of the mean time-correlation at t^* ,

$$\text{relative error} = \frac{p(z_n) \sigma_{z_n}}{\sqrt{w_n} C(t^*)} \quad (3.21)$$

The resulting distribution allows for quickly visualizing which z_n bins contribute the most uncertainty to the resulting time-correlation function and may require additional sampling. Plots of the relative error for each membrane system are provided in Figure B.7 in Appendix B. Also provided are the assessments of error in the committor time-correlation functions and permeability of the C36 (Figure B.8) and Drude DLPC (Figure B.9) systems before and after additional sampling is used.

The error in the permeability was estimated as follows. We estimate the global variance in the permeability according to eq 3.16, where the permeability is substituted for the value of the time-correlation function within $\sigma_{z_n}^2$ given by eq 3.17, such that,

$$\sigma_{z_n}^2 = \frac{1}{w_n} \sum_{i_n=1}^{w_n} \left(\frac{1}{\pi(-L/2)} \dot{c}_{i_n}(t^*; z_n) \right)^2 - \left(\frac{1}{w_n} \sum_{i_n=1}^{w_n} \frac{1}{\pi(-L/2)} \dot{c}_{i_n}(t^*; z_n) \right)^2 \quad (3.22)$$

where $\dot{c}_{i_n}(t^*; z_n)$ is estimated based on the slope of the best fitting straight line to the long lag time regime of the time-correlation function of a single trajectory, specifically from 300 to 400 ps. The uncertainty in the permeability is then the square root of the value given by

eq 3.16 (after substituting $\sigma_{z_n}^2$ with eq 3.22). This uncertainty is intended to estimate the error in the derivative of the long lag time regime of the time-correlation function, which is effectively the uncertainty in the plateau value of the permeability.

3.3.3 *All-atom MD simulation protocol*

In order to thoroughly characterize the membrane permeation of sucrose, a total of four systems were considered: DOPC and DLPC membranes, each modeled with additive and polarizable force fields. The additive force field is CHARMM36,^{101;102;103} and the polarizable force field is Drude2023.^{104;30} The MD was conducted with OpenMM 7.7⁸² using the Langevin integrator with 1 ps^{-1} damping at a temperature of 303 K. For the Drude simulations, the Drude particle thermostat was given a damping of 20 ps^{-1} and a temperature of 1 K. For both systems, the timestep was set to 1 fs for a more fair comparison. Constant pressure was maintained at 1 atm with the MonteCarloMembraneBarostat. For the additive systems, non-bonded interactions were treated with OpenMM VDW switching with a switching distance of 8 Å and cutoff distance of 12 Å. For the Drude systems, non-bonded interactions were treated with the LJ-PME method.³⁰ In the following analysis, reference to the collective variable z denotes the z -component of the center of mass distance between the heavy atoms of sucrose and the phosphorus atoms of the membrane. This value was computed using the CustomCentroidBondForce, and was output at a frequency of 1 ps.

3.3.4 *Enhanced sampling simulations*

For the additive systems, the initial membrane configurations, initial sucrose structure, and force field files were obtained from CHARMM-GUI.^{105;106} The initial additive hydrated membrane system was built with PACKMOL¹⁰⁷ by inserting a single sucrose into the center of each membrane and adding TIP3P water.¹⁰⁸ Each of these initial systems was simulated for 50 ns with a harmonic restraint on sucrose centered at $z = 0 \text{ Å}$ with a 5 kcal/mol Å^2

force constant. To generate the US windows, each sucrose was pulled in positive and negative directions in separate sets of steered-MD (SMD) simulations. Each sucrose was restrained with a force constant of $1 \text{ kcal/mol } \text{\AA}^2$, and center of the restraint was moved at a rate of $0.5 \text{ \AA/ns}$. Each SMD was run for 74 ns. Initial configurations for each window were saved from the SMD simulations every $1 \text{ \AA}$ along z from -36 to $+36 \text{ \AA}$, with two independent starting configurations for $z = 0 \text{ \AA}$. These coordinates were used as the initial windows for the additive systems. Using charmm modules,¹⁰⁹ each initial configuration was converted to the equivalent Drude representation, and the TIP3P water was replaced by SWM4.⁸⁰ The Drude windows were then minimized with the steepest descent algorithm as implemented in charmm. All of the windows for each membrane and force field were restrained with a force constant of $2.5 \text{ kcal/mol } \text{\AA}$ and simulated for 50 ns each. Before the analysis was conducted, the first 5 ns of each simulation were discarded to allow for equilibration.

The PMF of each system was computed with WHAM¹¹⁰ with $0.2 \text{ \AA}$ bin widths. The error bars were obtained based on the symmetry of the membrane, which involved using WHAM to independently compute the PMF from -36 to $-0 \text{ \AA}$ and from $+36$ to $+0 \text{ \AA}$. The errorbars are then set by $\pm \frac{1}{2} |W^{\text{plus}}(z) - W^{\text{minus}}(-z)|$. The position-dependent diffusivity was computed for each window using the method of Hummer.⁵ The upper limit of integration for the position autocorrelation function, while formally infinity, was set automatically for each window based on the condition that the autocorrelation function is less than 0.01 times the variance of z , or else set to a maximum of 2000 ps.³⁸ The PMF and position-dependent diffusivity are then plugged into the ISD model of eq 3.1 to obtain the permeability. More details about the calculation of position-dependent diffusivity are given in Appendix B, including the position autocorrelation function over a range upper limits of integration for several z windows in Figure B.10.

3.4 Results and discussion

3.4.1 *Validation of computational framework with BD stochastic simulations*

The validity of the expression for re-weighting the three time-correlation functions that we use for calculating permeability is established with 2-dimensional BD simulations with non-Markovian memory effects. The agreement between the permeability derived from the equilibrium time-correlation function and the corresponding re-weighted result shown in Figure 3.1C demonstrates the accuracy of the re-weighting scheme of eq 3.9. The strength of the approach is further supported by the equivalence of the time-correlation permeability and the counting permeability, or direct calculation of the net flux across the model membrane. Counting membrane crossings (in either direction) from long equilibrium trajectories can be converted to a permeability coefficient by,¹

$$P = \frac{N_c}{2t \pi(-L/2)} \quad (3.23)$$

where N_c is the number of crossings, t is the duration of the trajectory, and $\pi(-L/2)$ is the equilibrium probability at the edge of the membrane. The cutoff values dividing the membrane region from the bulk, or $\pm L/2$, were set to $z = \pm 35 \text{ \AA}$, where the PMF reaches a constant value (Figure B.1). The counting permeability is considered to be the authoritative estimate due to the directness of the calculation of the net flux across the membrane. We observe that the plateau value of each of the time-correlation derived permeability coefficients, from both the equilibrium and re-weighting, is in close agreement with the counting estimate (Figure 3.1C).

An important characteristic of the BD simulations that were constructed to test the time-correlation functions is that the ISD permeability value significantly underestimates

the exact permeability based on the net flux, owing to the system’s non-Markovianity and breakdown of the assumption of overdamped dynamics. The ISD permeability is obtained from eq 3.1, where the diffusivity is set to the minimum effective diffusivity based on the exponential memory function of the dynamics along z , which is $1/(1/D_z + 1/D_e)$.¹³ The ISD permeability is the theoretical lower limit of the overdamped GLE with exponential memory, which would be accurate for systems for which the timescale of the decay of the memory function is significantly faster than the timescale of the barrier crossing.

The equilibrium time-correlation functions of the committor (eq 3.5), indicator (eq 3.3), and indicator-restricted committor (eq 3.7) were computed from the ensemble of long equilibrium 2-dimensional BD stochastic simulations. The re-weighted time-correlation functions of each respective expression (eq 3.9) were computed from the ensemble of short BD trajectories. Each time-correlation function is shown in Figure 3.1A. The permeability coefficients associated with the time-correlation functions are shown in Figure 3.1C.

3.4.2 Membrane permeability of sucrose from enhanced-sampling simulations

The PMF and the position-dependent diffusivity of sucrose for each membrane and force field are shown in Figure 3.2. The effects of the composition and force field representation of each membrane system are qualitatively evident from the height and shape of each PMF. The PMFs are primarily necessary for obtaining the equilibrium probabilities for re-weighting via eq 3.9. Additionally, the PMFs and position-dependent diffusivity profiles are the two ingredients of the ISD model permeability (eq 3.1). The cutoff values for calculating the permeability, $\pm L/2$, were set to $z = \pm 36$ Å for each system, based on where the PMFs reach a constant value.

The permeability coefficients for each set of membranes and force fields were assessed in a way similarly to that of the BD simulations with non-Markovian effects. In this case, how-

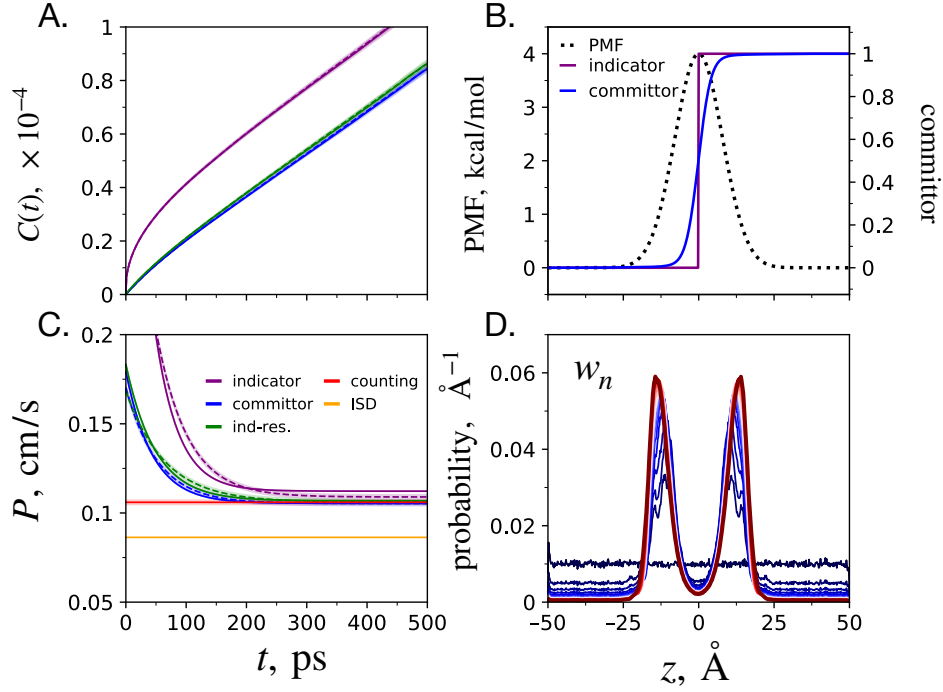


Figure 3.1: Results of BD stochastic simulations with non-Markovian memory effects. **A.** Indicator (purple), committor (blue), and indicator-restricted committor (green) time-correlation functions computed directly from equilibrium (dashed) with eq 3.5, eq 3.3, and eq 3.7, and by re-weighting short trajectories (solid) with eq 3.9. The error bars for the re-weighted time-correlation functions are $\pm$ the standard error via eq 3.16. The error bars for the equilibrium trajectories are $\pm$ the standard error of 593 replicate simulations. **B.** The PMF (dotted, black), indicator function (solid, purple), and approximate committor function (solid, blue) that are associated with this system. The cutoff values of $\pm L/2$ for the permeability calculations were set to $z = \pm 35$ Å. **C.** Permeability via eq 3.4, eq 3.2, and eq 3.6 from the equilibrium simulations (dashed) and from re-weighting short trajectories (solid). Also shown are the counting (red) and ISD (orange) values from eq 3.23 and eq 3.1, respectively. The counting permeability and errorbars are based on the mean and standard error of the number of crossings from 593 equilibrium trajectories. **D.** The normalized mean distribution of initial positions from 50 replicates of 20 iterations each of the optimization scheme of eq 3.20. The distributions are colored from dark blue (initial), to white (midpoint), to dark red (final).

ever, we have not attempted to simulate long equilibrium trajectories due to the constraint of computational cost. We therefore rely only on the three re-weighted time-correlation functions (eq 3.9) and the ISD model (eq 3.1) to estimate the permeability of sucrose. The re-weighted time-correlation functions computed from the sets of short unbiased trajectories of sucrose are shown in the top panels of Figure 3.3 for C36 and Figure 3.4 for Drude, while corresponding permeability coefficients are shown in the bottom panels. It is expected that time-correlation permeability will be greater than the ISD estimate if the memory function

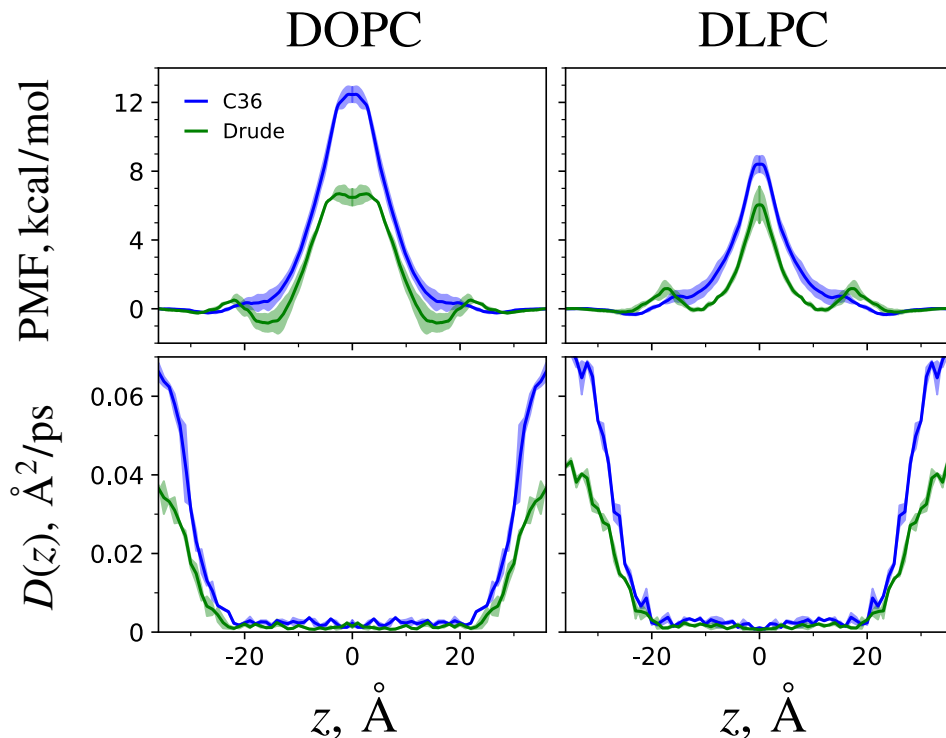


Figure 3.2: PMF (top row) and position-dependent diffusivity of sucrose across DOPC (left column) and DLPC (right column) membranes with the C36 additive (blue) and Drude2023 polarizable (green) force fields computed by umbrella sampling at 303 K. The errorbars are calculated based on symmetry: $\pm \frac{1}{2} |W^{\text{plus}}(z) - W^{\text{minus}}(-z)|$ for the PMF and similarly for the diffusivity.

decays slowly relative to the rate of barrier crossing, or equal to the ISD estimate if the memory function decays quickly. There are differences in the behavior of each time-correlation function between systems. In general, the committor time-correlation function converges the most smoothly and reaches a constant slope at the shortest lag time. The indicator-restricted committor time-correlation function behaves similarly to the committor correlation, but is perhaps more noisy. In most cases, the indicator time-correlation function, while eventually reaching the same constant slope as the other two, requires a longer lag time to do so. The main exception regarding the indicator time-correlation function is for the system of DOPC with Drude. For this system, the indicator permeability has a significantly larger plateau than the committor and indicator-restricted committor. We observe that this system in particular has the smallest and broadest PMF, which corresponds to most gradually changing

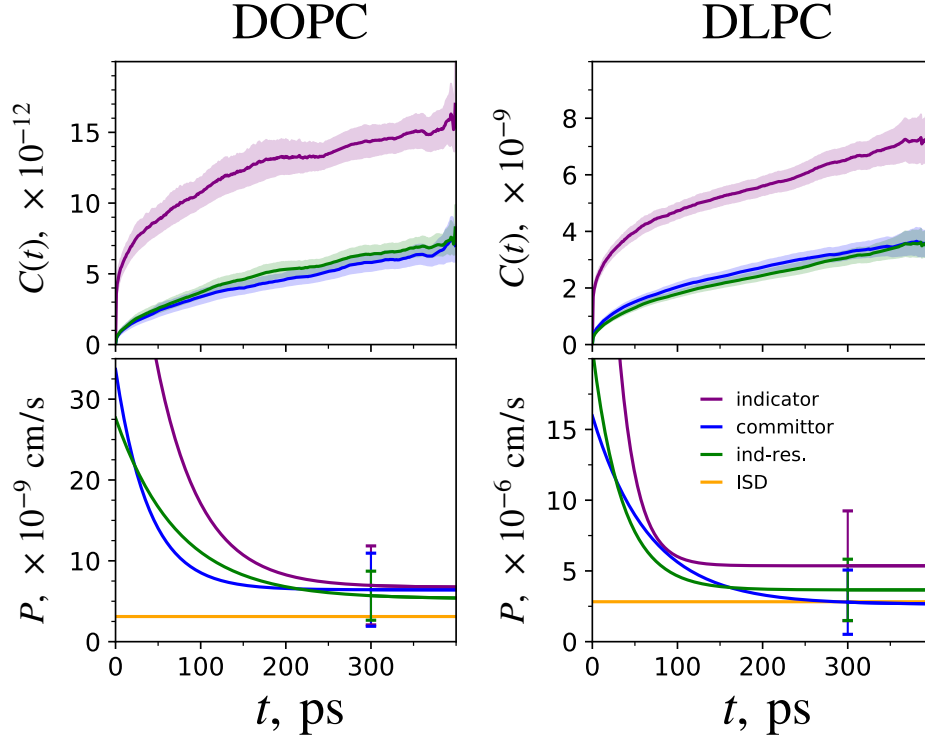


Figure 3.3: Results of sucrose membrane permeation MD simulations with the C36 force field for DOPC (left) and DLPC (right) membranes. Top: Indicator (purple), committor (blue), and indicator-restricted committor (green) time-correlation functions computed by re-weighting unbiased short trajectories via eq 3.9. The errorbars are $\pm$ the standard error via eq 3.16. Bottom: Permeability via eq 3.2, eq 3.4, and eq 3.6. Also shown are ISD (orange) values from eq 3.1 based on the US PMF and position-dependent diffusivity. The errorbars for permeability are $\pm$ the standard error via eq 3.16 and eq 3.22, based on the slope of the corresponding time-correlation function over the lag times $t^* = 300$ to 400 ps.

approximate committor via eq 3.8. The approximate committor for this system, therefore, has the largest deviation from the indicator function, which would lead to a longer relaxation time ($\tau_H > \tau_q$) before the constant slope is reached.¹⁸ We expect that extending each short trajectory by several hundred picoseconds and computing the indicator time-correlation function for longer lag times would result in a closer agreement committor permeability for this system. The behavior of the indicator compared to the committor time-correlation in the case of DOPC with Drude highlights the utility of the committor for minimizing the duration of each short trajectory needed for estimating the permeability. The approximate committor functions constructed via eq 3.8 that were applied for evaluating the committor time-correlation functions for each of the four systems are shown graphically in Appendix B.

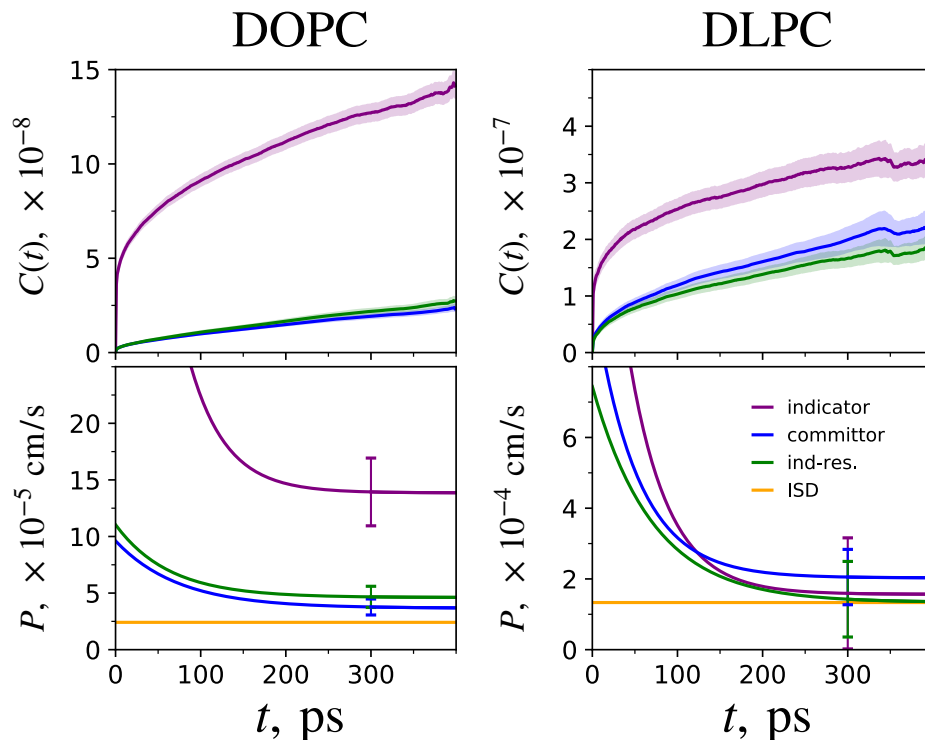


Figure 3.4: The same as Figure 3.3, except the Drude polarizable force field is used.

We consider the plateau values of the committor time-correlation function derived permeability to be our most reliable estimates of the true permeability, considering that these, as predicted by theory, have the smallest relaxation time, τ_q . These results are reported in Table 3.2. Considering first the additive force field, it is apparent that the permeability of DOPC to sucrose is significantly lower than that of DLPC. This result can be accounted for by membrane thickness, considering that sucrose is significantly solvated in the DLPC interior, but dry in DOPC.⁹⁶ For DOPC with the additive force field, the committor permeability is larger by about 2 times, while for DLPC they are nearly equivalent. The difference between membranes can be explained by the increased effective size of sucrose in DLPC due to hydration. The mean hydration numbers across each membrane system are provided in Figure B.11 in Appendix B. In the context of the memory function associated with the dynamics of each permeant inside the membrane, the larger committor permeability compared to the ISD model indicates that the dynamics projected onto z are not fully overdamped.

Pastor and coworkers observed that for water permeability simulations modeled with C36, the counting result is about 1.5 times larger than the ISD result for DOPC membranes, and about equal for DLPC membranes.¹ We consider the effect of polarizability on the permeation of sucrose by comparing the magnitude of the C36 permeability to the corresponding Drude permeability. Polarizability is shown by the permeability of the Drude systems to have significant consequences for the permeation of a highly polar molecule like sucrose. As has been demonstrated by Chen et al.⁹⁶ for charged molecules, the Drude force field has a lower free energy cost for desolvation. The Drude permeability results are orders of magnitude larger than the corresponding C36 permeability. Within the Drude representation, the increase in permeability from ISD to committor is a factor of 1.5 for both membranes.

Table 3.2: Sucrose permeability from MD simulations for DOPC and DLPC membranes, in units of $\times 10^{-6}$ cm/s

force field	lipid	ISD	committor
C36	DOPC	0.00311	0.00641 ± 0.0045
C36	DLPC	2.82	2.67 ± 2.3
Drude	DOPC	24.1	36.9 ± 7.1
Drude	DLPC	133	203 ± 78

Note: Committor permeability error is stderr via square root of variance obtained from eq 3.16 and eq 3.22.

We consider more closely the PMF and diffusivity for each system. The barrier heights for both membranes are greatly lowered by the polarizable force field. At $z = 0$, the PMF for DOPC decreased from 12.5 kcal/mol with C36 to 6.49 kcal/mol with Drude, and for DLPC it decreased from 8.40 kcal/mol to 6.05 kcal/mol. Notably, the barriers for each lipid were originally several kcal/mol apart with C36, but ultimately are within 0.5 kcal/mol of each other with Drude. These differences in PMF heights have a major impact on the resulting permeability for each force field. The lower barrier for polar molecule permeation with Drude compared to C36 is consistent with water permeability for a range of saturated and unsaturated lipids computed by Yu et al.³⁰ In addition to the barrier heights, we also

consider the diffusivity of sucrose at the center of each membrane. For DOPC the diffusivity is $0.0022 \text{ \AA}^2/\text{ps}$ with C36, and $0.0015 \text{ \AA}^2/\text{ps}$ with Drude. For DLPC, it is $0.00096 \text{ \AA}^2/\text{ps}$ with C36 and $0.00056 \text{ \AA}^2/\text{ps}$ with Drude. While Drude is expected to have a lower diffusivity than C36,⁸⁰ the reason for differences between each membrane modeled with the same force field is not immediately obvious. Because of its thickness, the center of the DOPC membrane is essentially dry. DLPC is significantly thinner, however, which results in hydration of sucrose even at the center, with a computed hydration number of roughly 3 to 6 waters. We note that the hydration number in the membrane is not greatly affected by the choice of force field. The larger effective size of sucrose inside the DLPC membrane compared to DOPC, therefore, accounts for the difference in diffusivity. The aqueous diffusivity of sucrose (D_{aq}), based on the $z = -36 \text{ \AA}$ region of DLPC in Figure 3.2, is approximately $0.073 \text{ \AA}^2/\text{ps}$ for C36 and $0.042 \text{ \AA}^2/\text{ps}$ for Drude. The decreased diffusivity of Drude over C36 by roughly a factor of 2 is consistent with what has been observed for water diffusivity between the two force fields.⁸⁰ The aqueous diffusivity of sucrose has been measured experimentally with spectrophotometry¹¹¹ and NMR,¹¹² also with MD simulations.¹¹² The C36 D_{aq} from this work is consistent with the previous MD study. The Drude D_{aq} is consistent with spectrophotometry experiments on sucrose which report $0.043 \text{ \AA}^2/\text{ps}$,¹¹¹ and similar to NMR experiments which report $0.0186 \text{ \AA}^2/\text{ps}$.¹¹²

3.4.3 *Comparison with experimental measurements*

Experimentally, sucrose permeability has been measured for several types of membranes, with a range of results (summarized in Table 3.3). We attempt to reconcile the sucrose permeability determined with MD simulations in this work with experimental results. Vesicle fluorescence permeability is a reasonable comparison for simulations, due to the ability of this experiment to model single-component membranes, as well as to alleviate complications such as unstirred layers. In the fluorescence quenching methodology of Poolman and co-

workers,^{22;113} the smallest detectable permeability of DOPC vesicles within the timescale of the experiments corresponded to zwitterionic amino acids, and were on the order of 10^{-9} cm/s. On this timescale, sucrose was impermeable, indicating that an upper bound on its permeability can be placed around 10^{-10} cm/s.²² This experimental result is about 64 times smaller than the C36 DOPC result, the smallest permeability obtained by our simulations. Alternatively, the permeability of DOPC to sucrose estimated by Seo et al.¹¹⁴ with PAMPA is around 3×10^{-8} cm/s, or about a factor of five larger than the C36 DOPC result, and about three orders of magnitude smaller than the Drude DOPC result. PAMPA results are generally not readily compared with MD due to confounding variables such as the unstirred layer, filter porosity, and nonpolar solvent used with the lipids.¹¹⁵ As an alternative experimental comparison, we consider two in vitro BBB model studies which found larger permeability than PAMPA.^{90;91} However, because BBB models use cell layers, there is a possibility of paracellular pathways. The permeability of the BBB to sucrose has medical significance. Isotopically labeled sucrose can be used as test of BBB damage, because it is neutral, stable in the blood, and is not known to interact with BBB membrane proteins.^{91;116} BBB permeability could potentially be a relevant comparison to MD simulations for these reasons, as well as the BBB's known small paracellular permeability.⁹⁰ It is possible, however, that the paracellular contribution may not be negligible for a molecule with a small enough permeability. BBB permeability to sucrose has been measured both in vivo and in vitro.^{91;90} Due to the presence of sucrase in Caco-2 cells, permeability estimates using this cell line are likely to be unreliable.¹¹⁷ To our knowledge, MD simulations of sucrose permeability have not previously been published.

One aim of this work was to investigate the validity of using sucrose as an inert osmolyte in vesicle permeability experiments, a role for which it is commonly employed.^{118;29} It is generally assumed that membranes are virtually impermeable to sucrose on the timescale of vesicle experiments. This assumption is supported by experimental evidence, for example

Table 3.3: Experimental sucrose permeability

membrane	$P_{\text{app}}, \times 10^{-6} \text{ cm/s}$
DOPC vesicle ^{113;22}	< 0.0001
PAMPA DOPC ¹¹⁴	0.0334
BBB ⁹¹	0.312
BBB ⁹⁰	1.886

Notes: The DOPC vesicle permeability was not explicitly reported, however, we assume that it is less than the smallest permeability determined in the study.²²

Diamond and Katz’s series of studies which claimed that the partition coefficient of sucrose in a membrane is undetectable,¹¹⁹ negative, or approximately zero.¹²⁰ From this perspective, it is apparent that the Drude permeability results obtained in this paper are too large, while the C36 results seem fairly realistic, presenting the future direction of continuing the refinement of the Drude polarizable force field for lipids and carbohydrates. This conclusion is overly simple, however, because we have exposed at least one cancellation of errors for C36 due to its overestimation of the diffusivity of sucrose in water, while Drude has very good agreement.

The interaction between sucrose and lipid membranes is complex. Since Cass and Finkelstein,³ experimental evidence has suggested an incompletely characterized interaction between sucrose and lipid membranes. More recently, Zong et al.⁹³ tested explicitly the osmolyte dependence of the permeability of DMPC giant unilamellar vesicles (GUVs) to water, finding a smaller permeability in the presence of glucose and sucrose compared to salts, attributed to deformation of the GUVs. A complete explanation of this effect requires characterizing how sucrose affects the physical properties of the membrane. The interaction of sucrose and similar disaccharides (such as trehalose) with lipid vesicles has been investigated with dynamic light scattering,¹²¹ SANS,⁸⁹ Raman spectroscopy, and other experimental methods.¹²² Early MD simulations suggested that sucrose dehydrates lipid headgroups.¹²³

Other MD work demonstrated that the addition of trehalose results in increased area per lipid.¹²⁴ There have also been attempts to calculate partial potentials of mean force (PMFs) of disaccharides near the headgroup region of lipid membranes with MD simulations.^{94;95} MD simulations designed to compare the interaction of sucrose with hydrated and dehydrated membranes have also been conducted.¹²⁵ Until now, however, a thorough study of the PMF, diffusivity, and permeability of sucrose across membranes has been lacking.

3.5 Concluding Discussion

Having the ability to rigorously treat slowly permeating molecules with MD simulations is critical for the development of a complete theory of membrane permeation. We have demonstrated the calculation of the permeability coefficient of sucrose by extracting time-correlation functions that are connected to the steady-state flux through transition path theory. The key to applying this method to such a system for which it is infeasible to obtain sufficient sampling with equilibrium MD is shown to be short unbiased trajectories with re-weighting based on equilibrium probabilities obtained from the PMF. This method for computing the permeability from enhanced sampling MD is more accurate than the classic ISD model in cases for which the assumption of overdamped dynamics is not strong.

After first validating the method for re-weighting unbiased short trajectories with equilibrium probabilities on stochastic BD simulations with non-Markovian memory effects, we calculated the permeability of membranes of different saturation to sucrose with both an additive (C36) and polarizable (Drude2023) force field. We observe that for all four of the systems considered here, the ISD permeability consistently yielded a smaller permeability than that derived from the re-weighted time-correlation functions. Within the framework of the generalized Langevin equation, a more accurate representation of the dynamics of a molecule in a membrane involves a memory function. When the memory function associated with the dynamics projected onto the z -coordinate decays slowly relative to the rate

of barrier crossing, the exact permeability is expected to be larger than the ISD result. We therefore conclude that dynamics along z are not fully overdamped for sucrose. Compared to our previous work with the committor time-correlation permeability for water,¹³ the deviation from the ISD model for sucrose is considerably smaller. This finding is in accord with the theories presented here, such that smaller molecules will have a more slowly decaying memory function in the membrane.

The permeability of sucrose is interesting in itself, considering its regular use as an inert osmolyte in vesicle experiments, its function as a cryoprotectant of membranes, and its application as a permeability marker of the blood-brain barrier. Our results indicate that sucrose’s permeability depends on both the membrane’s saturation and polarizability of the force field: in general the polarizable model yields higher permeability, and also the permeability is larger for the thinner, saturated membrane. While the permeability coefficients obtained for the additive force field are in better agreement with available experimental results, we do not assume that polarizable force field is unrealistic. The aqueous diffusivity of sucrose with the polarizable model agrees significantly better with experiment than the additive model. Also, it is known that water permeability agrees better with experiment when the polarizable model is used. It is expected that further refinement of Drude2023 for lipids and carbohydrates will continue to improve the accuracy of permeability calculations.

While the time-correlation derived permeability presented in this work is more accurate than the ISD model for cases in which dynamics in the membrane are not overdamped, there are cases of permeation, such as for charged molecules, in which the membrane itself is significantly deformed. In these cases, the assumptions of Green-Kubo linear response would not be expected to hold, indicating that a level of theory that incorporates more than simply dynamics along z is required.

CHAPTER 4

DEEP LEARNING OF POSITION-DEPENDENT DIFFUSIVITY FROM UMBRELLA SAMPLING MOLECULAR DYNAMICS SIMULATIONS

ABSTRACT – A first-line permeability tool is the inhomogeneous solubility-diffusion (ISD) model. A useful approximation, it requires two ingredients: the potential of mean force, which is well-defined, and the position-dependent diffusivity, which is often problematic. Here, a reconciliation of the Woolf-Roux and Hummer position-dependent diffusivity profiles extracted from umbrella sampling is achieved through deep learning memory functions in the time domain. The diffusivity extracted through this analysis is shown to be in best agreement with the equilibrium counting permeability for the same membrane system compared to both the Woolf-Roux and Hummer diffusivity. The effect of memory on the rate of barrier crossing is assessed through numerical simulations of the generalized Langevin equation (GLE). The GLE is efficiently simulated via Markovian embedding, which relies on the positive, decaying exponential form of the memory functions extracted by deep learning. Our results based on the ISD permeability, the known permeability from equilibrium MD, and the numerical GLE simulations indicate that memory effects most likely do not have a significant effect on the permeation of water.

4.1 Introduction

A widely used computational approach to calculate the permeability coefficient of a small molecule across a membrane using molecular dynamics (MD) simulations is the inhomogeneous solubility-diffusion (ISD) model, first derived by Diamond and Katz,⁸

$$P = \left[\int_{-L/2}^{L/2} dz \frac{e^{W(z)/k_B T}}{D(z)} \right]^{-1} \quad (4.1)$$

where L is the width of the membrane, z is the z -component of the distance between the permeant and the center of the membrane, $W(z)$ is the potential of mean force (PMF), and $D(z)$ is the position-dependent diffusivity. Part of the appeal of the ISD approximation is its synergy with MD simulations, which can be used to determine both the PMF and position-dependent diffusivity. Indeed, one of the earliest studies of water permeation through lipid membranes based on MD simulations relied upon this model.⁶ It is not without limitations, however. While the PMF can be determined using enhanced sampling techniques such as umbrella sampling,¹⁰ estimating the position-dependent diffusivity can be more challenging.^{126;49;127} For a molecule in a membrane (permeant), the diffusivity is expected to vary along z due to the inhomogeneous membrane environment. Therefore, estimating the diffusivity from the classic mean-square deviation (MSD) relation, $D = \lim_{t \rightarrow \infty} \langle (z(t) - z(0))^2 \rangle / 2t$, while valid for homogeneous bulk solution, is not strictly applicable in this case.

The friction coefficient, γ , is related to the diffusivity D via the Einstein relation, $D = k_B T / \gamma$. The friction can be estimated from the integral of the force-force time-correlation function exerted on a permeant *fixed* at position z .¹²⁸ However, this approach is not always reliable, as it has been shown to significantly overestimate the diffusivity in the case of an ion in water.¹²⁹ Alternatively, the permeant may be harmonically *restrained* around a position z to determine a local $D(z)$, although a special considerations are necessary to extract the diffusivity from the biased dynamics of the restrained molecule. For instance, the integral of the velocity-velocity time-correlation function, which is related to the diffusivity in homogeneous bulk solution according to Green-Kubo linear response, does not provide a useful route when there is a restraining potential.

The generalized Langevin equation (GLE) provides the most rigorous framework to pursue this type of analysis.¹⁴ From this mathematical construct, one may estimate the friction from the integral of the memory function, and the diffusion coefficient from the Einstein

relation. In fact, the treatments based on a *fixed* or *restrained* permeant are closely related. Berne et al. showed that the memory function of a particle *restrained* by a harmonic potential in the limit of an increasing spring constant asymptotically approaches the force-force time-correlation function of a *fixed* particle.¹³⁰ Ultimately, it is important to keep in mind that the physical picture of overdamped diffusive motion is valid only when certain fundamental conditions are met.^{131;132} In particular, the mass of the solute must be larger than the mass of the surrounding solvent, a condition that may or may not be satisfied as the permeant diffuses along z .

Despite these fundamental limitations, a practical strategy for extracting diffusivity from umbrella sampling simulations with a harmonic restraining potential can be formulated within the GLE framework. An approach based on the velocity-velocity time-correlation function was first derived by Woolf and Roux⁸⁴ following an analysis by Straub et al.¹³³ This result was revisited by Hummer to derive a mathematically equivalent form in terms of the position-position time-correlation function.⁵ This latter method is perhaps more widely used, but it is not necessarily more accurate in practical applications.^{1;126;13} It was shown by Rowley and coworkers that the Woolf-Roux and Hummer diffusivity expressions can yield significantly different results using the same set of trajectory data, and both can be unreliable.¹²⁶ Nevertheless, the origin of these unresolved problems has not been fully elucidated.

To circumvent these issues, we implemented a deep learning approach to directly extract the memory function of the GLE from umbrella sampling MD simulations. The deep learning approach, developed by Russo et al.,¹³⁴ employs a multilayer perceptron (MLP) to parameterize a sum of positive, decaying exponential functions that correspond to the memory function of a GLE in a harmonic potential in the time domain. The method is applied to the case of water permeation across a DLPC membrane. The memory function extracted by the MLP independently in each umbrella window was built into an effective position-dependent memory function across the membrane. After parameterizing the PMF

and the memory function, the position-dependent diffusivity across the membrane was determined and inserted into eq 4.1 to obtain the permeability. It is shown that the MLP is able to reliably extract the memory function of a water molecule across the highly inhomogeneous environment of a lipid bilayer. Using these results, the issues with the Woolf-Roux and Hummer treatment of the GLE are clarified. Lastly, the Grote-Hynes theory of barrier crossing¹⁶ is used to show that the physical picture of overdamped diffusive motion is valid for water permeation.

4.2 Theoretical developments

The physical model underlying the present analysis begins by representing the dynamics in terms of the generalized Langevin equation (GLE),¹⁴

$$m\ddot{z}(t) = -\frac{\partial W}{\partial z} - \int_0^t M(t-t') \dot{z}(t') dt' + f(t) \quad (4.2)$$

where m is the mass of the permeant, $W(z)$ is the potential of mean force (PMF), $M(t)$ is the memory function, and $f(t)$ is a Gaussian random noise obeying the second fluctuation-dissipation theorem, $\langle f(0)f(t) \rangle = k_B T M(t)$. By construction, the total friction given by

$$\gamma = \int_0^\infty M(t) dt \quad (4.3)$$

and the diffusivity D is recovered via the Einstein relation,

$$D = \frac{k_B T}{\gamma} \quad (4.4)$$

In the case of umbrella sampling simulations, the system is restrained by a harmonic potential, $k z^2/2$, with force constant k . In practice, the technical challenge consists in accurately extracting the memory function $M(t)$ from the biased dynamics of a permeant that is re-

strained around a specific position z .

4.2.1 Multilayer perceptron (MLP) position-dependent diffusivity

To treat the data from the umbrella sampling simulations, we reduce the GLE to time-correlation functions by multiplying both sides of eq 4.2 by the initial velocity, $\dot{z}(0)$, which results in,

$$C_{Fv}(t) = \int_0^t M(t-t') C_{vv}(t') dt' \quad (4.5)$$

where $\langle f(t)\dot{z}(0) \rangle = 0$, $C_{vv}(t) = \langle \dot{z}(t)\dot{z}(0) \rangle$ is the velocity autocorrelation function, and,

$$C_{Fv}(t) = -m\langle \ddot{z}(t)\dot{z}(0) \rangle - k\langle z(t)\dot{z}(0) \rangle \quad (4.6)$$

is the force-velocity time-correlation function.

To solve for the memory function of the permeant in a given umbrella window, we employ the multilayer perceptron (MLP) method developed by Russo et al.¹³⁴ The MLP method assumes that the memory function can be expressed as a sum of positive, decaying exponential functions, where each exponential term is linked to a unique neuron during optimization. In the MLP method, the memory function is given by,

$$M(t) = \sum_{i=1}^N K_i e^{-t/\tau_i} \quad (4.7)$$

where N is the total number of neurons, and K_i and τ_i are the parameters to be optimized. The coupling constants, K_i , are related to the mean-square fluctuation of the force acting on the permeant molecule, which can give rise to “caging” effects in the diffusive dynamics.¹³⁵ In the Markov limit, each exponential component contributes to the total friction according

to eq 4.3, yielding

$$\gamma = \sum_{i=1}^N \gamma_i \quad (4.8)$$

with $\gamma_i = K_i \tau_i$. Following the Einstein-Smoluchowski relation (eq 4.4), the effective diffusivity for the memory function of eq 4.7 may be expressed as,

$$D^{MLP} = \left[\sum_{i=1}^N \frac{\gamma_i}{k_B T} \right]^{-1} \quad (4.9)$$

The optimization of the MLP proceeds by iteratively minimizing a loss function based on the mean-square error between the current estimate of and the known value of $C_{Fv}(t)$. Here, we expand the original definition of the MLP loss function of Russo and coworkers¹³⁴ for the memory function optimization. In our approach, we implement a loss function that incorporates additional information about the memory function, in particular an estimate of the value of its integral, via the Einstein-Smoluchowski relationship to the diffusivity (eq 4.4). The Woolf-Roux (eq 4.13) and Hummer (eq 4.15) diffusivity serve as guidelines to establish a reasonable range for the integral of the predicted memory function. When the the modified loss function is applied, the resulting value of eq 4.9 is effectively constrained by a flat-bottomed harmonic function based on the Woolf-Roux and Hummer diffusivity estimates for each umbrella window, such that

$$\begin{aligned} \mathcal{L} = & \frac{1}{\mathcal{T}} \sum_{n=0}^{\mathcal{T}-1} (C_{Fv}(n\Delta t) - C_{Fv}^{MLP}(n\Delta t))^2 \\ & + \frac{1}{2} \left(\frac{1}{D^H} (D^{MLP} - D^H) \right)^2 \theta(D^H - D^{MLP}) \\ & + \frac{1}{2} \left(\frac{1}{D^{WR}} (D^{MLP} - D^{WR}) \right)^2 \theta(D^{MLP} - D^{WR}) \end{aligned} \quad (4.10)$$

where $C_{Fv}^{MLP}(t)$ is the convolution integral of the known $C_{vv}(t)$ with the current estimate

of $M(t)$, $\theta(x) = 1$ if $x > 0$ and 0 otherwise, and $\mathcal{T}\Delta t$ is the maximum lag time to be calculated, after which it is assumed $C_{Fv}(t)$ and $C_{vv}(t)$ have fully decayed. D^{WR} is the Woolf-Roux diffusivity and D^H is the Hummer diffusivity, which effectively act as upper and lower bounds on eq 4.9, respectively. As an initial justification for the choice of D^{WR} as the upper bound and D^H as the lower bound in eq 4.10, we draw on the findings of Rowley and coworkers¹²⁶ which indicate that the diffusivity estimated by Woolf-Roux is generally larger than that estimated by Hummer.

A comparison of the MLP's convergence for the original (simple) and modified loss functions is given in Figure C.1 of Appendix C, including the simple loss function's apparent failure to obtain a reasonable estimate of the position-dependent diffusivity. The convergence of each window is assessed by the mean-square error (MSE) between the known $C_{Fv}(t)$ and the $C_{Fv}^{MLP}(t)$ predicted by the convolution integral of the memory function and the velocity autocorrelation function. A crucial observation is that for the center region of the membrane ($|z| < 3$ Å), the MSE is at a minimum compared to the rest of the membrane, indicating successful optimization. In addition, the resulting diffusivity estimates for this region are in very good agreement between the two loss functions. This finding indicates that the flat-bottomed harmonic loss function does not affect MLP estimates that naturally converge to values in between the Woolf-Roux and Hummer boundaries.

4.2.2 *Position-dependent diffusivity*

Traditionally, the position-dependent diffusivity has been extracted from biased umbrella sampling MD simulations by solving for the Laplace transform of the memory function. It is assumed that, in the presence of the biasing potential, the system undergoes small fluctuations $\delta z(t)$ around the mean value position $\langle z \rangle$. To treat the data from the biased simulation, we reduce the GLE to time-correlation functions associated with the local behavior around the mean position $\langle z \rangle$ by multiplying both sides of eq 4.2 by the initial velocity, $\delta \dot{z}(0)$ (or

equivalently $\dot{z}(0)$), which results in,

$$m \dot{C}_{vv}(t) = -\frac{k_B T}{\langle \delta z^2 \rangle} \langle \delta z(t) \delta \dot{z}(0) \rangle - \int_0^t M(t-t') C_{vv}(t') dt' \quad (4.11)$$

where $k_B T / \langle \delta z^2 \rangle = (W'' + k)$ is the second derivative of the total potential, $C_{vv}(t) = \langle \delta \dot{z}(t) \delta \dot{z}(0) \rangle$, $\dot{C}_{vv}(t) = \langle \delta \ddot{z}(t) \delta \dot{z}(0) \rangle$, and $\langle \delta z(t) \delta \dot{z}(t) \rangle = \int_0^t C_{vv}(t) dt$. The Laplace transform of the memory function, $\tilde{M}(s)$, is related to the diffusivity via,

$$D = \lim_{s \rightarrow 0} \frac{k_B T}{\tilde{M}(s)} \quad (4.12)$$

Correspondingly, we can also define $D = \lim_{s \rightarrow 0} \tilde{D}(s)$. The so-called Woolf-Roux diffusivity ascribed to the position $\langle z \rangle$ is given by,⁸⁴

$$D^{WR} = \lim_{s \rightarrow 0} \left[\frac{-\tilde{C}_{vv}(s) \langle \delta z^2 \rangle \langle \delta \dot{z}^2 \rangle}{\tilde{C}_{vv}(s) [s \langle \delta z^2 \rangle + \langle \delta \dot{z}^2 \rangle / s] - \langle \delta z^2 \rangle \langle \delta \dot{z}^2 \rangle} \right] \quad (4.13)$$

where $\tilde{C}_{vv}(s)$ is the Laplace transform of the velocity autocorrelation function. Because of the singularity located at $s = 0$, it is necessary to extrapolate from a well-behaved region of $\tilde{D}(s)$ back to zero to obtain the limit in eq 4.13. The Hummer diffusivity approach begins with a substitution of $\tilde{C}_{vv}(s)$ by the Laplace transform of the position autocorrelation function in eq 4.13.⁵ In Laplace space, the relationship between these two time-correlation functions is given by,

$$\tilde{C}_{vv}(s) = -s^2 \tilde{C}_{zz}(s) + s \langle \delta z^2 \rangle \quad (4.14)$$

where $C_{zz}(t) = \langle \delta z(t) \delta z(0) \rangle$ is the position autocorrelation function and $\tilde{C}_{zz}(s)$ is its Laplace transform. The Hummer diffusivity is obtained by substituting eq 4.14 in eq 4.13 in the limit

of $s \rightarrow 0$,⁵

$$\begin{aligned}
D^H &= \lim_{s \rightarrow 0} \frac{\langle \delta \dot{z}^2 \rangle \langle \delta z^2 \rangle (\langle \delta z^2 \rangle - s \tilde{C}_{zz}(s))}{\tilde{C}_{zz}(s) [s^2 \langle \delta z^2 \rangle + \langle \delta \dot{z}^2 \rangle] - s \langle \delta z^2 \rangle^2} \\
&= \lim_{s \rightarrow 0} \frac{\langle \delta z^2 \rangle^2}{\tilde{C}_{zz}(s)}
\end{aligned} \tag{4.15}$$

The final expression can also be written as $\lim_{t \rightarrow \infty} \int_0^t C_{zz}(t') dt'$ in the time domain.⁵

In either approach, a reliable numerical evaluation of the expression requires a careful examination of its convergence in relation to the limiting process. Complete derivations of the memory functions in Laplace space for the Woolf-Roux and Hummer methods are provided in the appendix, and also in the appendix of the work by Rowley and coworkers.¹²⁶ eq 4.14 is of central importance for understanding the differences and similarities of the two approaches. This expression relates the Laplace transform of the velocity-velocity time-correlation function used in the Woolf-Roux method and the position-position time-correlation function used in the Hummer method.

4.3 Computational details

4.3.1 Molecular dynamics simulations

The details of the set of umbrella sampling MD simulations of water in a DLPC membrane that were employed for the extraction of the position-dependent diffusivity with the Woolf-Roux, Hummer, and MLP approaches are as follows. The membrane and water systems each consisted of 72 monomers of 1,2-dilauroyl-*sn*-glycero-3-phosphocholine (DLPC) lipids and 2880 water molecules, represented by the Drude2023 lipid force field³⁰ and SWM4,⁸⁰ respectively. The umbrella sampling simulations were conducted in triplicate, with a unique initial configuration for each replicate of each window. The initial configurations were generated from the trajectory of a spontaneous water crossing event from three independent

equilibrium simulations, which were conducted in our previous work.¹³ The harmonic restraints were spaced by 1 Å from $z = -30$ to $+30$ Å for a total of 61 windows per replicate set of simulations. The force constant (k) of each harmonic potential was set to 10 kcal/mol Å². We note here that one of the umbrella sampling replicates was conducted for our previous work,¹³⁶ and two additional replicates were generated for the present work. Each window for each set of umbrella sampling simulations was run for 30 ns, for a total of 5490 ns of sampling. When computing the time-correlation functions for all of the following analysis, the first 5 ns of each trajectory were excluded to allow for equilibration. The value of the collective variable z was recorded every time step (1 fs).

The $C_{Fv}(t)$ and $C_{vv}(t)$ time-correlation functions were computed for each replicate of each window along z separately. Each was computed up to a lag time of 15 ps, with increments of 1 fs. The instantaneous velocity and acceleration were evaluated by finite-difference.

The key MD protocol is as follows. The MD engine OpenMM version 7.7⁸² on GPU with the Nose-Hoover integrator was used with a timestep of 1 fs, temperature of 303 K, friction of 10 ps⁻¹, chain length, number of steps, and Yoshida-Suzuki all set to 3. The Drude particles were set to a temperature of 1 K and a friction of 1 ps⁻¹. LJ-PME was used for non-bonded interactions with a cutoff of 10 Å. The simulations were run at a constant volume of $47.3 \times 47.3 \times 70.8$ Å ($x \times y \times z$). For convenience, the OpenMM front-end rickflow was used.⁵²

4.3.2 Numerical simulation of the GLE

Due to spatially varying friction across the membrane environment, it is expected that the memory function obtained within each umbrella sampling window will have position-dependent parameters, $K_i(z)$ and $\tau_i(z)$. The overall position-dependent memory function is modeled as,

$$M(t; z) = \sum_{i=1}^N K_i(z) e^{-t/\tau_i(z)} \quad (4.16)$$

where N was chosen to be 2. The GLE with position-dependent memory is obtained by substituting eq 4.16 into eq 4.2 ,^{137;138}

$$\begin{aligned}
m\ddot{z}(t) = & -\frac{\partial W(z)}{\partial z} \\
& - \int_0^t \sum_{i=1}^N K_i(z(t')) e^{-(t-t')/\tau_i(z(t'))} \dot{z}(t') dt' \\
& + f(t)
\end{aligned} \tag{4.17}$$

This GLE with position-dependent memory is an approximation,^{139;137;138} although it will be shown to be a good representation for water permeability through a lipid membrane.

We adapted a numerical integration scheme provided by Bond and coworkers¹⁵ for the simulation of the GLE with the position-dependent memory function composed of a sum of positive, decaying exponential terms (Prony series) via Markovian embedding. The form of the memory function assumed by the MLP of Russo et al.¹³⁴ is well-suited for the numerical integration schemes developed by Bond and coworkers.¹⁵ The Markovian embedding scheme adapted to accommodate the position-dependent parameters of the memory function yields,¹⁵

$$\dot{z}(t + \Delta t) = \dot{z}(t) - \frac{\Delta t}{m} \frac{\partial W(z)}{\partial z} + \frac{\Delta t}{m} \sum_{i=1}^N S_i(t) \tag{4.18}$$

$$z(t + \Delta t) = z(t) + \Delta t \dot{z}(t + \Delta t) \tag{4.19}$$

$$\begin{aligned}
S_i(t + \Delta t) = & S_i(t) - \frac{\Delta t}{\tau_i(z)} S_i(t) - \Delta t \frac{\gamma_i(z)}{\tau_i(z)} \dot{z}(t + \Delta t) \\
& + \frac{1}{\tau_i(z)} \sqrt{2k_B T \gamma_i(z) \Delta t} \mathcal{N}_t
\end{aligned} \tag{4.20}$$

where Δt is the timestep, $z(t)$ is the main reaction coordinate, $S_i(t)$ is an embedded Ornstein-Uhlenbeck process that depends on $\tau_i(z)$ and the friction $\gamma_i(z) = K_i(z) \tau_i(z)$ in the i th term of the memory function, and $\mathcal{N}_t$ is a normally distributed random number.

We tested and compared two Markovian embedding integration schemes developed by

Table 4.1: Equilibrium GLE simulations

integrator	landscape	# trajectories	time/ jectory (μ s)	tra- jectory (μ s)	total (μ s)	time
EM-3	zero mean force (0 kcal/mol)	500	0.05		25	
EM-3	half PMF (3 kcal/mol)	500	1		500	
EM-3m	zero mean force (0 kcal/mol)	500	0.05		25	
EM-3m	half PMF (3 kcal/mol)	500	1		500	
EM-3m	full PMF (6 kcal/mol)	1000	10		10000	

Bond and coworkers,¹⁵ each based on the Euler-Maruyama integrator. In the original study, the Euler-Maruyama integrator that demonstrated the best stability for a GLE with a position-independent memory function was called “method 3.” We will refer to this integrator as EM-3. They also developed a multi-stage splitting variation of EM-3 with twice as many velocity (4) and position (2) updates per step, inspired by Leimkuhler and Matthews.¹⁴⁰ We will refer to this multi-stage splitting integrator as EM-3m. These two schemes were assessed for their stability and accuracy with respect to the mitigation of spurious drift due to the position-dependence of the parameters of the memory function.

The position-dependent memory functions were constructed based on the output of the MLP optimization for each window of the umbrella sampling MD simulations along z . Because the MLP was implemented with two neurons, each umbrella window yielded two optimized values each for K_i and τ_i . The assignment of $i = 1$ or $i = 2$ for a given window by the MLP is arbitrary with respect to the other windows. To ensure the stability of the numerical integration and to better visualize the physical significance of each term in the memory function, the larger τ_i were assigned to $i = 1$, and the smaller τ_i were assigned to $i = 2$. To simplify the implementation and maximize computational efficiency, the each term ($i = 1, 2$) in the position-dependent memory function was expressed as a set of $K_i(z)$ and $D_i(z) = k_B T / K_i(z) \tau_i(z)$, and fit to analytical functions by visual inspection and least-squares.

Test simulations of the GLE were conducted first to assess the importance of numerical issues arising from the implementation of the position-dependent memory function, which corresponds to nonlinear friction in the framework of the standard GLE.^{137;141;142} The position-dependent memory function leads to a spurious drift that is comparable to that of Brownian dynamics integrated with a position-dependent diffusivity.¹⁴³ This effect is also present in Langevin dynamics simulations with position-dependent friction due to the Itô vs. Stratonovich dilemma.^{144;145} The validity of the integration scheme was assessed by evaluating the error in the free energy landscape sampled by the simulations with respect to the forces used in the underlying GLE. We did not seek to implement an exact GLE with nonlinear friction, such as was done by Ayaz et al.¹³⁸

The set of equilibrium GLE simulations run for this study is summarized in Table 4.1. The set of equilibrium simulations cover three different free energy landscapes. To test the stability and accuracy of the EM-3 and EM-3m integration schemes, a zero mean force system was simulated. The zero mean force simulations were also used to calculate the position-dependent diffusivity via mean-square deviations (MSD) in displacements. To ensure efficient sampling of the entire z -coordinate, each trajectory was initialized from a random z . As an additional test case to compare the two integrators, a system with a mean force corresponding to a barrier of 3 kcal/mol was run. In the presence of the barrier, the trajectories were initialized in the bulk. The superior accuracy of EM-3m over EM-3 is established in Figure C.2 of Appendix C. The main set of equilibrium GLE simulations used the EM-3m integrator and the full PMF of water across the DLPC membrane based on the 1-D probability distribution from equilibrium MD simulations. For computational efficiency, an analytical function was fit to the PMF that was extracted from the MD simulations. The analytical derivative of the fitted PMF was used for calculating forces in the numerical simulations. For all of the equilibrium GLE simulations, the temperature was set to 303 K, the timestep was 1 fs, and the z coordinate was saved every 1 ps. To confine the system,

upper and lower half harmonic walls with a force constant of 5 kcal/mol $\text{\AA}^2$ were applied to the particle if it diffused outside of $\pm 50 \text{ \AA}$.

In addition, GLE umbrella sampling simulations with the full PMF of water were carried out to directly examine the accuracy of the Woolf-Roux and Hummer estimates of the diffusivity. As in the all-atom MD, the harmonic restraints were spaced by 1 $\text{\AA}$ from $z = -30$ to $+30 \text{ \AA}$ for a total of 61 windows, and the force constant of each harmonic potential was set to 10 kcal/mol $\text{\AA}^2$. Each window for each of the three sets of umbrella sampling simulations was run for 30 ns, for a total of 5490 ns of sampling. The calculation of the time-correlation functions used the same protocol as the MD simulations.

4.3.3 Determination of the diffusivity

MLP method. The MLP was implemented following the details described by Russo et al.¹³⁴ The optimization is conducted for a single umbrella window at a time. The MLP consisted of three layers: input, hidden, and output. Two neurons were chosen for the hidden layer. The input layer contains a time-axis up to 15 ps with 1 fs spacing, corresponding to the same shape as the time-correlation functions from the MD simulations. The input layer is connected to each neuron by edges with weights and biases. For the activator function of each neuron of the hidden layer, the input from the previous layer is exponentiated and then time-convolved with the known $C_{vv}(t)$ using the convolution function of TorchAudio.¹⁴⁶ Each neuron in the hidden layer is connected to the output layer by edges with weights (but no biases). The current estimate of $C_{Fv}(t)$ is then returned via the weighted sum of the output of each neuron, which was used to evaluate the loss at each iteration. To build the neural network, PyTorch was used.¹⁴⁷ The optimizer used was Rprop, which is the same one used by Russo et al.¹³⁴ The optimization of the memory function proceeded for 500 epochs.

To ensure that the memory function is strictly a sum of positive, decaying exponential functions, the weights of the input layer are constrained to be negative, while the weights of

the output layer are constrained to be positive. The weights of input layer are initialized to be the negatives of exponentiated Gaussian random numbers. The biases of the input layer are initialized to be Gaussian random numbers. The weights of the output layer are initialized to be exponentiated Gaussian random numbers. At each iteration, it was necessary to calculate the current estimate of the diffusivity for evaluating the modified loss function with respect to the upper and lower bounds imposed by Woolf-Roux and Hummer. The parameter $1/\tau_i$ is the negative of the i th weight of the input layer. The parameter K_i is the i th weight of the output layer times the exponentiated bias of the input layer. The effective diffusivity can then be computed with eq 4.9, and the relationship $\gamma_i = K_i\tau_i$. The structure of the MLP is shown in Figure C.3 in the Appendix C. eq C.2 is an expression for the diffusivity in terms of the weights and biases of the model at a given iteration of optimization.

Woolf-Roux method. The Laplace transform of $C_{vv}(t)$ was calculated by numerical integration with the trapezoid rule with $ds = 0.0001 \text{ ps}^{-1}$ up to $s = 1.5 \text{ ps}^{-1}$. At each s , the integration was performed over the entire 15 ps that was computed for $C_{vv}(t)$ with $dt = 0.001 \text{ ps}$. After calculating $\tilde{D}(s)$ according to eq 4.13 up to $s = 1.5 \text{ ps}^{-1}$, the $s = 0$ value was determined by the y -intercept of a straight line fit by least-squares to an automatically determined set of points. The set of points for fitting depended on the presence of a singularity (besides $s = 0$) in the range of s being considered. If the singularity was detected, the fitting was inspired by the method of Rowley and coworkers.¹²⁶ The line was fit to a set of 20 points (20 ds) centered on the point of least curvature, determined by where the finite-difference second derivative of $\tilde{D}(s)$ is closest to zero. In cases where there was no singularity detected, the function generally passes through an inflection point and then increases approximately linearly. The line for this case was fit to all of the points past the point of greatest negative curvature (the minimum of the finite-difference second derivative).

Hummer method. The position autocorrelation function was computed up to a maximum time of 500 ps for each replicate of each window with an increment 1 fs. The time

integral of each position autocorrelation function was computed with the trapezoid rule with $dt = 0.001$ ps. In order to obtain the best estimate of the diffusivity (without including unnecessary noise in the integral of the position autocorrelation function), the upper limit of integration (t_∞) was optimized for each window and replicate independently. The optimization of t_∞ was inspired by a method employed by Dickson et al.³⁸ The upper limit of t for a window centered at z is set by the point where $0.01 \times \langle \delta z^2 \rangle > C_{zz}(t)$, but not greater than 500 ps.

4.4 Results

4.4.1 *Extraction of the position-dependent memory function with the MLP*

The parameters K_i and τ_i of eq 4.7 were optimized by the MLP for each window of the umbrella sampling simulations of water in a DLPC membrane. It is assumed that the memory function can be accurately modeled with two positive, decaying exponential terms. Several memory functions that were optimized by the MLP in distinct regions of the membrane are shown in Figure 4.1. The position-dependence of the parameters of the memory function is apparent in the visually distinct behavior of each region. For instance, the optimized memory function at $z = 15$ Å is shown to decay relatively slowly compared to those of the $z = 0$ Å center of the membrane and the $z = 30$ Å bulk region. The position-dependence of the MLP optimization is further illustrated by the lag time dependent functions that compose the GLE of eq 4.5 in different regions of the membrane. The velocity autocorrelation functions for every umbrella window are shown in Figure C.4, the force-velocity autocorrelation functions are shown in Figure C.5, and the optimized memory functions are shown in Figure C.6 in Appendix C. From the bulk solution to the center of the membrane, the timescale of decay and shape of each time-correlation function gradually changes.

The optimized parameters of the position-dependent memory function across the mem-

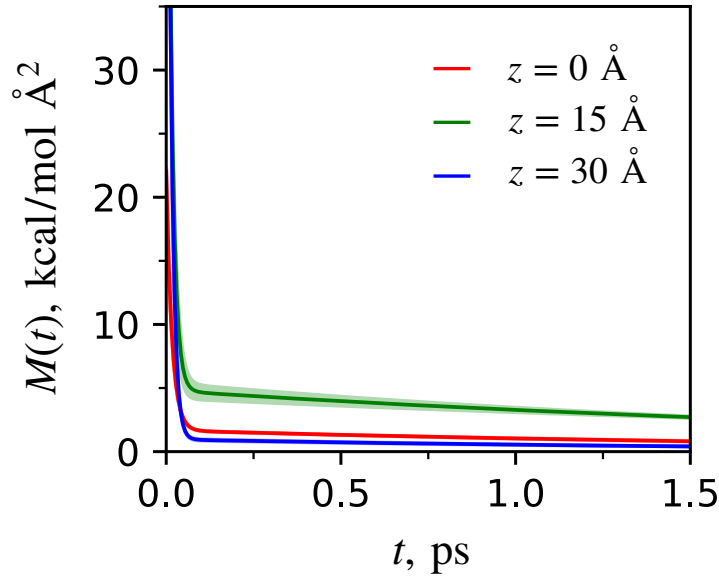


Figure 4.1: Optimized memory functions, $M(t)$, extracted from three distinct regions of the umbrella sampling MD simulations of water in the membrane: $z = 30$ (blue), $z = 15$ (green), and $z = 0$ Å (red). The uncertainty intervals are given by plus and minus the standard deviation of the three replicates.

brane are shown in Figure 4.2. The distinct timescales of decay for each term ($i = 1, 2$) are revealed by the plots of $\tau_i(z)$ in Figure 4.2B. From the set of $\tau_i(z)$ and $K_i(z)$, the effective friction coefficients associated with each term i can be resolved, which are shown in Figure 4.2C. A physical picture of the GLE emerged after the MLP distinguished two regimes for the terms in the memory function. The first is the existence of a slowly decaying term that dominates the effective diffusivity ($i = 1$) and a second contribution that is quickly decaying ($i = 2$). Notably, the friction associated with the slower $i = 1$ mode, when expressed as $k_B T / \gamma_1(z)$, accounts almost entirely for the effective position-dependent diffusivity. These sets of position-dependent parameters, extracted from MD umbrella sampling simulations, can be used to construct a memory function for a GLE that can be efficiently simulated with numerical integration. It is interesting to note from Figure 4.2 that the coupling factors $K_i(z)$ display a significant dependence on the position z , whereas the relaxation times $\tau_i(z)$ are nearly constant over z . This observation suggests that there are two dominant natural

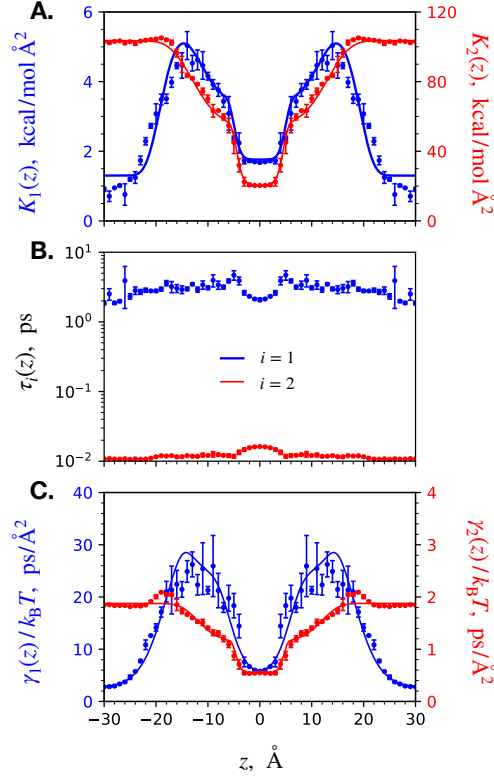


Figure 4.2: The position-dependent parameters of the optimized memory function (eq 4.7) extracted by the MLP with $N = 2$ with the modified loss function from umbrella sampling MD simulations of water in a DLPC membrane. **A.** The coupling factor, $K_i(z)$, **B.** the timescale of decay, $\tau_i(z)$, and **C.** $\gamma_i(z)/k_B T$ for each term the the $i = 1$ (blue) and $i = 2$ (red) terms of the optimized memory function. The dots correspond to the mean of three independent simulations with error bars given by plus and minus the standard deviation of each quantity. The lines for $K_i(z)$ and $\gamma_i(z)/k_B T$ correspond to analytical functions which were fit to the points by visual inspection and least-squares, and subsequently used for the numerical integration of the GLE.

relaxation modes within the bilayer, with relaxation times τ_1 and τ_2 . These relaxation times are inherent features of the membrane fluctuations. On the other hand, the strength of the coupling of the permeant molecule with these two modes is strongly dependent on position.

A constructive explanation of this observation may be drawn from a spectral decomposition of the effective dynamical propagator of the entire system^{148;18} under the assumption that the natural fluctuations of the membrane are not perturbed significantly by the presence of the permeant molecule at position z . For a fixed molecule, the memory function is related to equilibrium time-correlation function of the force exerted by the membrane in the

z direction, $\langle \delta F_z(t) \delta F_z(0) \rangle$, with $\delta F_z = F_z - \langle F_z \rangle$. The time-correlation function can be expressed as,^{148;18}

$$\langle \delta F_z(t) \delta F_z(0) \rangle = \sum_{i>0} (\delta F_z \cdot \phi_i^R)^2 e^{-t/\tau_i} \quad (4.21)$$

with

$$(\delta F_z \cdot \phi_i^R) = \int d\mathbf{x} \delta F_z(\mathbf{x}) \phi_i^R(\mathbf{x}) \quad (4.22)$$

where $\mathbf{x}$ represents the coordinates of the system, $\phi_i^R(\mathbf{x})$ is the i th right-eigenmode of the effective dynamical propagator of the system with its associated eigenvalue e^{-t/τ_i} , the equilibrium distribution corresponds to the first right-eigenvector $\phi_0^R(\mathbf{x})$, and $\langle F_z \rangle = (F_z \cdot \phi_0^R)$. According to the MLP analysis, the sum over i is dominated by the contribution from two eigenmodes. The friction is then,

$$\begin{aligned} \gamma(z) &= \frac{1}{k_B T} \int_0^\infty \langle \delta F_z(t) \delta F_z(0) \rangle dt \\ &= \frac{1}{k_B T} \sum_{i>0} (\delta F_z \cdot \phi_i^R)^2 \tau_i \\ &= \frac{1}{k_B T} (\delta F_z \cdot \phi_1^R)^2 \tau_1 + \frac{1}{k_B T} (\delta F_z \cdot \phi_2^R)^2 \tau_2 + \dots \\ &\approx K_1(z) \tau_1 + K_2(z) \tau_2 \end{aligned} \quad (4.23)$$

The coupling factors $K_i(z)$ are related to the mean-square fluctuations of the force exerted on the permeant projected onto the i th right-eigenmode ϕ_i^R of the effective dynamical propagator, $\langle \delta F_z \delta F_z \rangle = \sum_{i>0} (\delta F_z \cdot \phi_i^R)^2$. Based on the MLP analysis, it appears that the relaxation time of the first two eigenmodes, τ_1 and τ_2 , do not depend strongly on the position z . The prefactors $(\delta F_z \cdot \phi_i^R)^2$, however, vary with z . This outcome is conceivable if the fixed permeant molecule can be treated as a perturbation and the dominant contribution to

$\gamma(z)$ arises from the natural eigenmodes of the unperturbed membrane.

4.4.2 *Validation of the numerical integration of the GLE*

Having extracted the position-dependent memory functions from the umbrella sampling MD simulations, it is possible to proceed with the numerical simulations of the GLE. Comparison of two integration schemes for modeling the GLE with a position-dependent memory function confirms the superiority of the multi-step approach of EM-3m. We suspect that the strength of EM-3m is related to the extra position update within a timestep, which allows for smoother integration of the position-dependent friction, $\gamma_i(z)$, and the coupling factors, $K_i(z)$. A comparison of the EM-3 and EM-3m simulations is shown in Figure C.2 of Appendix C. Our first assessment of integrator accuracy is based on simulations with no PMF. The absence of a PMF allows for the evaluation of the mean-square deviation (MSD) diffusivity and the detection of spurious drift due to integration errors. The MSD diffusivity extracted from this test shows virtually no difference between the EM-3 and EM-3m integrators (shown for EM-3m in Figure 4.3A). It has the expected behaviors of both position and lag time dependence. For the short lag time of 5 ps, the diffusivity profile overestimates the effective diffusivity of the underlying GLE. By 15 ps, however, there is good agreement, indicating that approximately this lag time is required for the full friction to accumulate. The spurious drift was computed based on the 1-dimensional probability distribution, $p(z)$. The “drift” PMF for these simulations can be determined directly from the marginal distribution as, $-k_B T \ln[p(z)]$. Comparison shows that deviations from the expected flat free energy are smaller for the EM-3m integrator than the EM-integrator. As an additional assessment, each integrator was tested on a 3 kcal/mol barrier (referred to as the “half PMF” system). The drift PMF in this case is the difference between the $-k_B T \ln[p(z)]$ extracted from each simulation and the analytical PMF input. In the presence of the half PMF, the EM-3m again has a smaller magnitude of spurious drift, demonstrating its superiority in modeling

the position-dependent memory function.

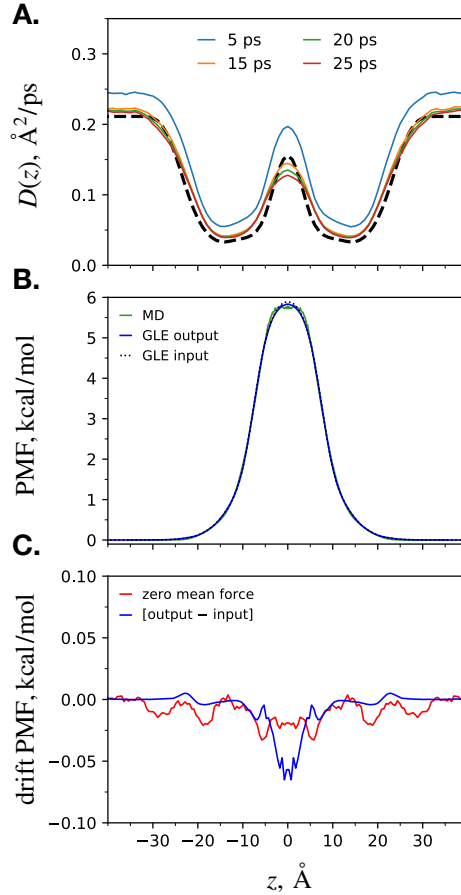


Figure 4.3: Assessment of the EM-3m numerical integration scheme for the GLE with the position-dependent memory function and the PMF of water extracted from MD simulations of water across a DLPC membrane. **A.** MSD $D(z)$ on flat free-energy surface (zero mean force) for several lag times. The exact diffusivity of the underlying GLE (dashed black line). **B.** The PMF of water in a DLPC membrane from the marginal probability distribution of unbiased MD simulations (green line). The PMF modeled by an analytical function used to compute the underlying mean force of the GLE (dotted black line). The PMF computed from the 1-D probability distribution of the equilibrium GLE simulations with the EM-3m integrator (blue line). **C.** The PMF of the zero mean force GLE simulation, or drift PMF (red line). The difference between the 1-D probability distribution PMF and the analytical input from the equilibrium GLE simulations (blue line). The PMFs are symmetrized about $z = 0$.

Finally, the accuracy of the EM-3m integrator was assessed based on the GLE simulations used to model the permeation of water. The PMF of water extracted from the equilibrium MD simulations was fit to an analytical function for efficiency. The two PMFs, shown in Figure 4.3B, are essentially equivalent. For comparison, the PMF calculated from $p(z)$ from the long equilibrium GLE simulations is also shown. The deviations are, again, very small.

The drift PMF for quantifying the integration error of EM-3m with the full PMF of water is shown in Figure 4.3C. This difference has a magnitude up to 0.065 kcal/mol at the very center for the full PMF, compared to fluctuations of around several hundredths of a kcal/mol for the zero mean force simulation. The integration error is sufficiently small for the purposes of estimating rates of barrier crossing with the numerical GLE simulations. On the basis of these results it can be concluded that an accurate coarse-grained representation of the the permeation of water molecules through a DLPC membrane is provided by the GLE.¹⁴⁹

4.4.3 *Determination of the position-dependent diffusivity*

The position-dependent diffusivity extracted with the Woolf-Roux (eq 4.13), Hummer (eq 4.15), and MLP (eq 4.5) approaches from the biased umbrella sampling simulations of the all-atom DLPC membrane are displayed in Figure 4.4. The Woolf-Roux diffusivity is consistently larger than the Hummer diffusivity for the entire range of z across the membrane. The effect of the modified loss function (eq 4.10) on the MLP diffusivity for a given window is highly dependent on the window’s location in the membrane. Near the bulk solution ($|z| > 20$ Å), the Woolf-Roux and Hummer diffusivity are in good agreement, and the MLP tends towards the slightly lower boundary. In the lipid tails region ($3 < |z| < 20$ Å), the MLP converges to the upper limit, as the difference between Woolf-Roux and Hummer widens. At the center of the membrane ($|z| < 3$ Å), the MLP converges to an intermediate value. This region also exhibits the largest difference between Woolf-Roux and Hummer. The three $z = 0$ values of the diffusivity are 0.235 ± 0.035 , 0.0841 ± 0.0052 , and 0.1583 ± 0.0069 Å²/ps for Woolf-Roux, Hummer, and MLP, respectively. The center of the membrane is the peak of the PMF, and therefore has the most critical impact on the resulting ISD permeability estimate.

Because the three approaches attempt to solve the GLE for the diffusivity based on the same set of trajectory data, an explanation of the deviations between each is warranted. First of all, the diffusivity from the Woolf-Roux method is sensitive to the procedure used for

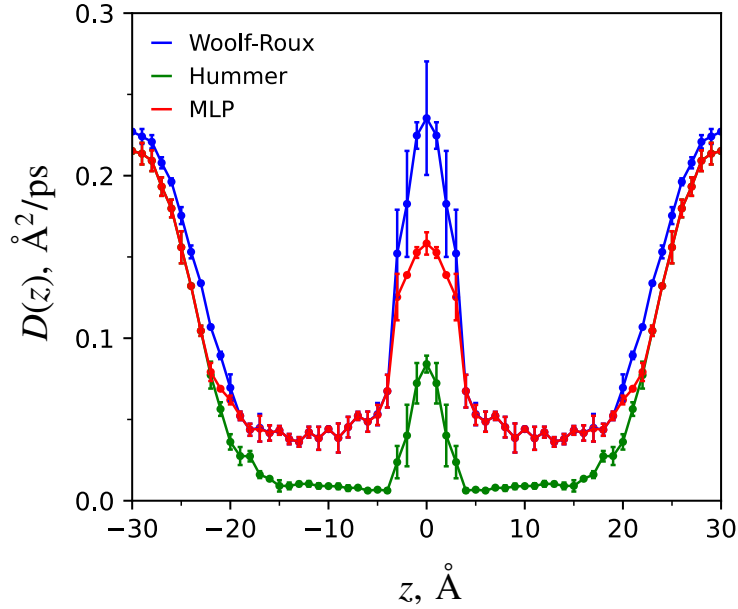


Figure 4.4: Position-dependent diffusivity ($D(z)$) from umbrella sampling MD simulations of water across a DLPC membrane computed with Woolf-Roux (blue), Hummer (green), and MLP (red) approaches. The plotted values are the mean of the three replicates for each window, which were first symmetrized about $z = 0$. The localized error bars are plus and minus the standard deviation between the three replicates.

extrapolating the Laplace transform of the diffusivity to zero. The difficulty with extracting the diffusivity in terms of the Laplace transform of the velocity autocorrelation function is apparent upon inspection of eq 4.13, where there is a singularity when $s = 0$. Detailed plots of these extrapolations are available in Figure C.7, Figure C.8, and Figure C.9 in Appendix C. Hummer has a relatively well-behaved diffusivity in Laplace space at $s = 0$, which is apparent in eq 4.15, where the diffusivity can be computed via the integral of the position autocorrelation function in the time domain. However, the Hummer diffusivity can be sensitive to the upper limit of integration. The position autocorrelation function decays slowly, in some cases (around $z = \pm 15$ Å) it is still nonzero at a lag time of 500 ps. The running integrals of the position autocorrelation functions up to 500 ps for each window up are shown in Figure C.10 in Appendix C.

The MLP method is advantageous for several reasons. The first is that it is highly automated. It does not require the fitting procedure of Woolf-Roux due to its use of time-

correlation functions in the time domain. Second, it does not depend on the position autocorrelation function, such that the slow decay ambiguity is avoided. A limitation of the original MLP optimization in the time domain is that sometimes apparently degenerate solutions of the memory function are obtained, which may or may not provide reasonable estimates of the diffusivity. By imposing boundaries set by the physically solid Hummer and Woolf-Roux approaches via the modified loss function (eq 4.10), however, the MLP effectively incorporates the memory function’s integral, instead of strictly its time-dependent values, into the optimization.

To delve further into the origin of the deviations between the Woolf-Roux and Hummer methods, we consider the fundamental link between them within the framework of the GLE in a harmonic potential. In particular, Woolf-Roux depends on the velocity autocorrelation function, while Hummer depends on the position autocorrelation function. In Laplace space, these two can be interchanged with eq 4.14. In practice, this key relation is not strictly satisfied by finite sampling from a simulation. To examine the accuracy of eq 4.14, plots of the directly computed and formulaically interchanged position ($\tilde{C}_{zz}(s)$) and velocity ($\tilde{C}_{vv}(s)$) autocorrelation functions from several windows of the umbrella sampling MD simulations are shown in Figure 4.5 A. and B., respectively. Importantly, $\tilde{C}_{vv}(s)$ does not identically vanish at $s = 0$ based on the sampling of the MD simulation. On the other hand, this condition is automatically satisfied if $\tilde{C}_{vv}(s)$ is converted formulaically from the position autocorrelation function via eq 4.14. The corresponding Laplace transforms of the memory functions based on $\tilde{C}_{zz}(s)$ and $\tilde{C}_{vv}(s)$ are shown in Figure 4.5C. The directly computed $\tilde{C}_{zz}(s)$ memory function is effectively that of the Hummer formulation, while the directly computed $\tilde{C}_{vv}(s)$ memory function is effectively the Woolf-Roux formulation. As has been observed by others,¹²⁶ the Woolf-Roux memory function diverges at small s , while the Hummer memory function is well-behaved. Observing the behavior of the formulaically converted $\tilde{C}_{vv}(s)$ and $\tilde{C}_{zz}(s)$ reveals that it is not inherently the Woolf-Roux formulation

that leads to the characteristic divergence, but the initial calculation of $\tilde{C}_{vv}(s)$. When $\tilde{C}_{vv}(s)$ is converted formulaically to $\tilde{C}_{zz}(s)$, the Hummer memory function would also diverge at small s . Conversely, when $\tilde{C}_{zz}(s)$ is converted to $\tilde{C}_{vv}(z)$, the Woolf-Roux memory function would be well-behaved at small s , although the value of $\tilde{C}_{zz}(s)$ at small s becomes very important.

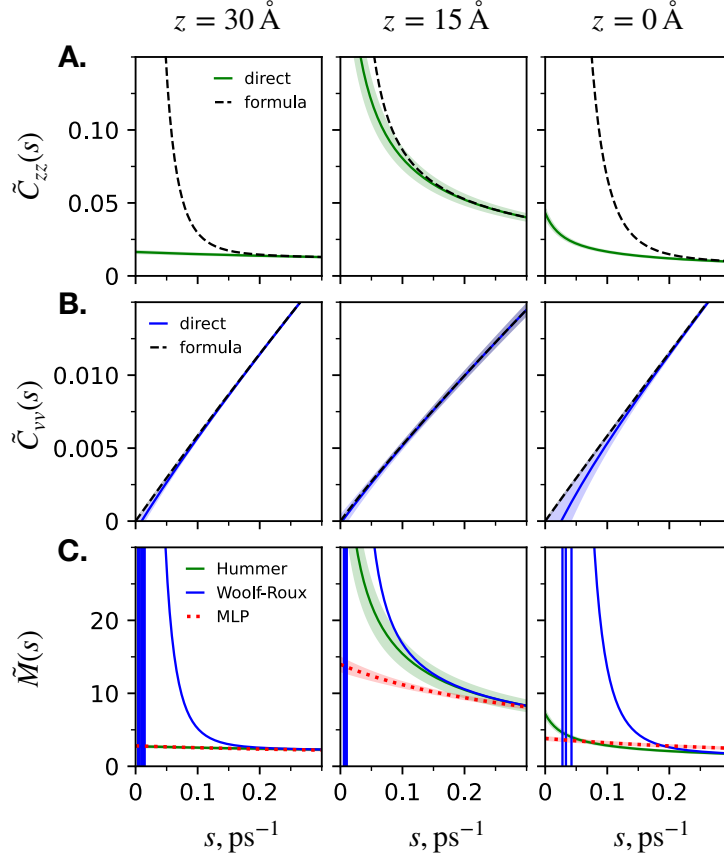


Figure 4.5: Examining the accuracy of eq 4.14 based on three representative windows of the umbrella sampling MD simulations ($z = 30, 15$ and $0 \text{ \AA}$). **A.** Laplace transform of the position autocorrelation function computed directly (solid green line) and converted from the velocity autocorrelation function via eq 4.14 (dashed black line). **B.** Laplace transform of the velocity autocorrelation function computed directly (solid blue line) and converted from the position autocorrelation function via eq 4.14 (dashed black line). **C.** Laplace transforms of the memory functions computed with the direct position autocorrelation functions, corresponding to the Hummer method (solid green line). Laplace transforms of the memory functions computed with the direct velocity autocorrelation functions, corresponding to the Woolf-Roux method (solid blue line). Laplace transforms of the MLP memory functions in Laplace space (eq 4.24), shown for reference (dashed red line). For each plot, the shaded regions correspond to uncertainty intervals based on the standard deviation between the three replicates.

We provide this analysis as an addition to the previously discussed discrepancies between Hummer and Woolf-Roux from Rowley and coworkers.¹²⁶ In their interpretation, it is the difference in timescales of the decay of the position-autocorrelation vs. velocity autocorrelation function that leads to the deviations in diffusivity. The position autocorrelation function, in certain membrane regions, may decay so slowly that it incorporates slow degrees of freedom that are not assumed by the underlying GLE. The Woolf-Roux method is expected to have higher accuracy due to the rapidly decaying velocity autocorrelation function. However, it has increased uncertainty at the critical small s region due to divergence of the memory function when $\tilde{C}_{vv}(s)$ does not identically reach 0 when $s = 0$. On the other hand, the MLP approach effectively regularizes the analysis by imposing an analytical form of the memory function (a sum of two decaying exponentials). It is interesting to include the novel MLP memory function in the discussion of the small s behavior. The Laplace transform of the MLP memory function (eq 4.7) is given analytically by,

$$\tilde{M}^{MLP}(s) = \sum_{i=1}^2 \frac{K_i}{s + 1/\tau_i} \quad (4.24)$$

For reference, the MLP memory function in Laplace space ($\tilde{M}^{MLP}(s)$) was plotted alongside the Hummer and Woolf-Roux memory functions in Figure 4.5. Due to the modified loss function, the $s = 0$ value is constrained to fall between those of Woolf-Roux extrapolated to $s = 0$ and Hummer at $s = 0$. Interestingly, for both $z = 0$ and $z = 15$ Å, $\tilde{M}^{MLP}(s)$ begins to deviate from the other two memory functions at approximately $s = 0.2$ ps⁻¹ (5 ps), where Woolf-Roux begins to diverge and experience high uncertainty. At the same time, it ultimately reaches $s = 0$ at a smaller y -intercept than Hummer, which we expect is due to the reliance of the MLP on the quickly decaying velocity autocorrelation function. Therefore, the MLP method effectively serves as an automatic extrapolation of Woolf-Roux to $s = 0$, while working in the time domain.

Lastly, we use the non-Markovian GLE simulations to further illustrate the convergence of the Woolf-Roux and Hummer approaches for determining the diffusivity. As with the MD simulations, we carried out biased umbrella sampling with the numerical GLE simulations. The simulations were conducted using the same PMF, position-dependent memory function, and EM-3m integrator as the long equilibrium GLE simulations. The umbrella sampling was designed to reproduce the biased MD simulations in terms of force constants, total sampling time, and system conditions. By this construction, the Woolf-Roux and Hummer approaches can be systematically checked for accuracy, because the exact diffusivity of the GLE is known.

The Woolf-Roux and Hummer position-dependent diffusivity computed for these simulations are shown in Figure 4.6. The agreement between Woolf-Roux and Hummer with the

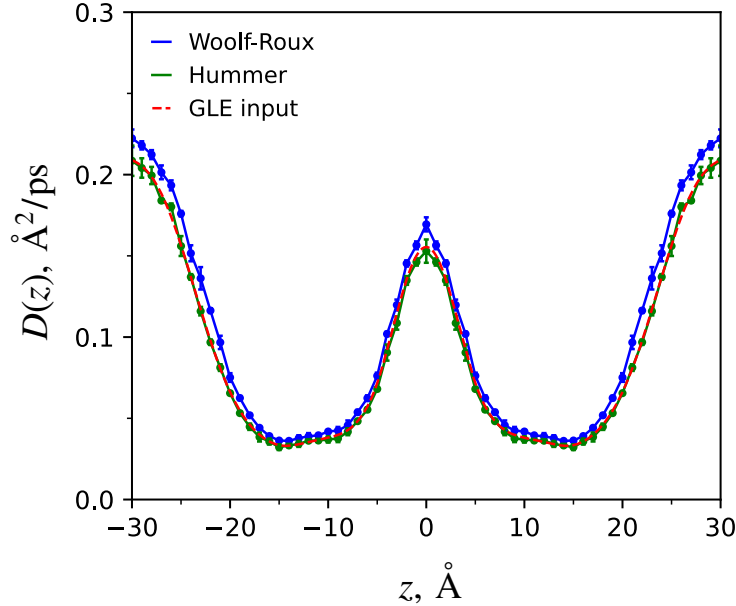


Figure 4.6: Position-dependent diffusivity ($D(z)$) from umbrella sampling numerical GLE simulations with the EM-3m integrator. The two main approaches, Woolf-Roux (blue) and Hummer (green), are used, showing strong agreement with each other. Also shown is the $D(z)$ of the underlying GLE (dashed red). The plotted values are the mean of the three replicates for each window, which were first symmetrized about $z = 0$. The localized error bars are plus and minus the standard deviation between the three replicates.

diffusivity input in the GLE. This is in contrast with the wide discrepancies observed from

the analysis of the all-atom MD simulations (Figure 4.4), especially at the critical $z = 0$ Å region. The Woolf-Roux is slightly larger than Hummer by a factor of 1.1 at this position, compared to a factor of 2.8 for the same point for the MD simulations. We have shown that both approaches can reliably extract the known position-dependent diffusivity of the underlying GLE. The extrapolation results for the Woolf-Roux diffusivity for each window are detailed in Figure C.11, Figure C.12, and Figure C.13, and the running integrals of the position autocorrelation functions in each window for the Hummer diffusivity are shown in Figure C.14 in Appendix C. For completeness, the velocity autocorrelation functions are shown in Figure C.15 and the force-velocity autocorrelation functions are shown in Figure C.16 in Appendix C.

This numerical test based on umbrella sampling GLE simulations provides further support to the conjecture that the primary source of disagreement between the velocity (Woolf-Roux) and position (Hummer) routes is based on uncertainty in extrapolating (for Woolf-Roux) and the overlap of the position autocorrelation function with slow degrees of freedom (for Hummer). When the numerical GLE is used to model the dynamics, there is no longer a possibility of interference by slow degrees of freedom, and the agreement between the velocity and position routes improves dramatically. This analysis has also supports that the fitting and extrapolation criteria for Woolf-Roux is reasonable.

4.4.4 *Evaluation of the permeability*

The most direct and straightforward treatment of permeation consists of counting spontaneous membrane crossing events during unbiased trajectories.^{50;1;52;13} Even for the GLE, this strategy remains critical, as there is no rigorous closed-form mathematical expression of the permeability coefficient for an arbitrary PMF and position-dependent memory function. For this reason, the permeability estimated from the long, equilibrium simulations serves as the reference for all of the other methods. The permeability based on counting membrane

crossings is given by,^{13;1;11}

$$P_{\text{counting}} = \frac{N_c(t)}{2t \pi(-L/2)} \quad (4.25)$$

where $N_c(t)$ is the total number of crossings in either direction up to time t , and $\pi(-L/2)$ is the equilibrium probability at the edge of the membrane. In our previous work, we estimated the counting permeability of the water and DLPC membrane system to be $8.83 \pm 0.79 \times 10^{-3}$ cm/s.¹³ Based on the 10,000 μs of sampling from the long, equilibrium GLE simulations, 295 crossings were observed in total. This rate of crossings corresponds a permeability of $9.96 \pm 0.61 \times 10^{-3}$ cm/s. The trajectories of all of the observed permeation events in the GLE simulations are shown in Figure 4.7. The beginning of each permeation was chosen

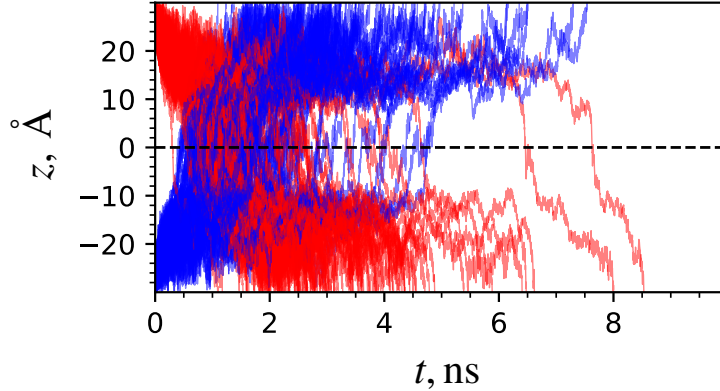


Figure 4.7: All of the reactive trajectories from the equilibrium numerical GLE simulations. Crossings from side a to side b are colored blue, and crossings from b to a are colored red. Based on 1000 independent simulations of 10 μs each, there was a mean of 0.295 crossings with a variance of 0.322, which is in line with the expected Poisson distribution. The mean permeability is 0.00996 cm/s with a standard error of 0.00061 cm/s. The mean reactive transit time, or mean of duration of each crossing, is 2.97 ns.

to be when the reactive trajectory leaves region a or b (when z becomes less than 30 Å or greater than -30 Å, respectively). Conversely, the end of the event is when the trajectory first touches the opposite side. Based on this analysis, the mean reactive transit time is then the average duration of each of the 295 permeation events, which was found to be 2.97 ns. Notably, this timescale is similar to that observed from the unbiased MD simulations

of water, which was 2.93 ns.¹³ This analysis shows that the GLE with position-dependent memory provides a physically sound coarse-grained representation of water permeability across a lipid membrane.

The permeability coefficients based on the various approaches are summarized in Table 4.2. The ISD permeability for the GLE system based on the analytical PMF and the exact position-dependent diffusivity of the underlying GLE is 9.38×10^{-3} cm/s. However, based on the PMF obtained from the $p(z)$ that is actually sampled in the long, equilibrium simulations, the ISD permeability is 10.0×10^{-3} cm/s. We can also assess the relative accuracy of each position-dependent diffusivity approach to estimate the permeability. The ISD model MD permeability estimates were obtained by using each position-dependent diffusivity (Woelf-Roux, Hummer, MLP) as an input in eq 4.1, in conjunction with the single PMF from the equilibrium simulations. Because the same PMF is used in each case, we are able to evaluate each of the position-dependent diffusivity profiles for accuracy. While the Hummer diffusivity significantly underestimates the counting permeability, Woelf-Roux is only slightly larger. The MLP is in closest agreement. We expect that its increased robustness and accuracy is due to a combination of the automated optimization, avoidance of the position autocorrelation function, and introduction of the modified loss function.

Table 4.2: Permeability of DLPC to water, units of $\times 10^{-3}$ cm/s

MD simulations		
	ISD, Woelf-Roux ¹	10.29 ± 0.20
	ISD, Hummer ¹	1.519 ± 0.074
	ISD, MLP ¹	8.72 ± 0.27
	counting ^{2,3}	8.83 ± 0.79
GLE simulations		
	ISD, input parameters	9.38
	ISD, output PMF ⁴	10.0
	counting ³	9.96 ± 0.61

Notes: 1. Uncertainty interval from standard deviation between three replicates of position-dependent diffusivity estimation. 2. Taken from ref¹³. 3. Uncertainty interval based standard error in the number of crossings from equilibrium simulations. 4. Permeability based on PMF from equilibrium $p(z)$ sampling and the analytical position-dependent diffusivity.

An intriguing question concerns the importance of memory effects on the permeability of water through a lipid membrane. An all-atom model of a lipid membrane is a molecular system exhibiting a complex dynamics. Yet, the strong agreement between the ISD and counting permeability from the long, equilibrium GLE simulation suggests that memory effects do not have a significant impact on the rate of barrier crossing. To explore this issue further, we exploit the Grote-Hynes theory designed to model the non-Markovian barrier crossing in transition rate theory.¹⁶ In this theory, the transmission coefficient, κ , which modulates the the rate from transition state theory, is given by, $\kappa = s/\omega_0$ where $\omega_0 = \sqrt{-W''/m}$, W'' is the (negative) second derivative of the inverted parabolic PMF, and s is the real solution of the equation,¹⁶

$$-W'' - m s^2 = s \tilde{M}(s) \quad (4.26)$$

Carrying out the Grote-Hynes analysis based on eq 4.26 with the MLP memory function yields a result corresponding to the non-Markovian dynamics of the GLE. On the other hand, neglecting the term ms^2 associated with inertial effects in eq 4.26 and replacing $\tilde{M}(s)$ by $\tilde{M}(0)$ yields a result that corresponds to the memory-less Markovian overdamped dynamics of the ISD approximation.¹³ By solving eq 4.26 numerically with the MLP memory function (eq 4.24), it is found that the ratio of the ISD permeability to the non-Markovian permeability is 0.96, which confirms that water permeation is not significantly affected by memory effects. The full Grote-Hynes analysis is illustrated in Figure C.17 and the results are collected in Table C.1 in Appendix C.

The physical reason is that the relaxation time over the inverted parabolic barrier is small compared to the short relaxation times of the memory function. This is explicitly shown starting from, $-W'' = s(\tilde{M}(0) + s\tilde{M}'(0) + \dots)$, and assuming that $\tilde{M}'(0)$ is small. To zeroth order, the situation reduces to the ISD approximation, $s_{\text{ISD}} = -W''/\tilde{M}(0)$. The Grote-Hynes solution accounting for memory effect is written in terms of a perturbation δs

relative to s_{ISD} . To lowest order, the ratio of ISD to GH permeability is,

$$\frac{P_{\text{ISD}}}{P_{\text{GH}}} \approx 1 - \frac{W'' \tilde{M}'(0)}{\tilde{M}(0)^2} = 1 - \frac{\tau_m}{\tau_b} \quad (4.27)$$

where $\tau_m = -\tilde{M}'(0)/\tilde{M}(0)$ is the mean relaxation time of the memory function, and $\tau_b = -k_{\text{B}}T/DW''$ is the relaxation time of the random motion over the energy barrier. In the present case, $\tau_m \ll \tau_b$. This finding is consistent with the good agreement between the ISD permeability and the counting permeability of the numerical GLE simulations. By extension, because the PMF and position-dependent memory function of the GLE system are quantitatively close to those of the MD system by construction, it is anticipated that memory effects have a negligible impact on water permeability through lipid membranes.

4.5 Conclusion

A theoretical framework based on the Generalized Langevin Equation (GLE) was used to examine the permeability of water molecules across lipid membranes. The multilayer perceptron (MLP) deep learning approach of Russo et al.¹³⁴ was extended to extract the position-dependent memory function from biased umbrella sampling MD simulations of the permeant molecule across the membrane. The memory function was modeled as a sum of two positive, decaying exponential terms. It was shown that the MLP method can reliably extract the diffusivity from MD simulations. This is in contrast with the familiar Woolf and Roux⁸⁴ and Hummer⁵ methods, which can display severe inaccuracies and numerical instabilities. One source of error associated with those methods appears to be the use of the Laplace transform of time-correlation functions to solve the GLE in closed form. In contrast, the present MLP approach solves for the memory function directly in the time domain. This procedure, together with the assumption of an analytical form of the memory function, provide a way to regularize the extraction of the space-dependent memory function and the diffusivity from

umbrella sampling simulations.

The GLE parametrized from the MD data could be numerically integrated efficiently with Markovian embedding, which was possible due to the assumed form of the memory function.¹⁵ A permeability of about 9.96×10^{-3} cm/s is estimated using unbiased simulations based on the GLE with a PMF and memory function extracted from all-atom MD simulations of water in a DLPC membrane. This result compares well with an estimate of 8.83×10^{-3} cm/s from all-atom MD simulations of a DLPC membrane.¹³ Furthermore, the mean reactive transit time from the all-atom MD simulations and the GLE simulations are very similar, which are 2.93 and 2.97 ns, respectively. These results indicate that the GLE with position-dependent memory provides a physically sound and accurate representation of water permeation across a lipid membrane. In addition, the ISD permeability estimate from MD (with the MLP diffusivity) ranges between 8.72 and 10.0×10^{-3} cm/s based on the MD and GLE simulations. The near equivalence of the ISD and counting estimate for the permeability, together with an analysis based on the Grote-Hynes theory of barrier crossing demonstrate that memory effects have a negligible impact on this example of a permeation process.

The validation of the ISD model for this system, in addition to the development of an improved estimation of the position-dependent diffusivity from umbrella sampling MD simulations, is promising for the future of permeability calculations. In the field of drug design, the screening of small molecules by permeability can depend critically on the ISD model. Rigorous validation of its assumptions remains significant as small molecule screening becomes more widespread and automated.^{20;150;151} In the future, we are interested in further assessing the accuracy of the numerical integration scheme described in this work for a GLE with position-dependent memory. It will be of interest to consider if more exact methods¹³⁸ will yield consistent results with the straightforward approach presented here. Future directions may also include tests of systems for which the ISD model is not valid, to determine if

the GLE parameterized by the MLP can effectively solve for the exact permeability.

4.6 Appendix: Derivation of the Woolf-Roux and Hummer diffusivity

Beginning with the time-correlation function representation of the GLE,

$$-m\langle\delta\ddot{z}(t)\delta\dot{z}(0)\rangle - K\langle\delta z(t)\delta\dot{z}(0)\rangle = \int_0^t M(t-t')\langle\delta\dot{z}(t)\delta\dot{z}(0)\rangle dt' \quad (4.28)$$

We substitute, $C_{vv}(t) = \langle\delta\dot{z}(t)\delta\dot{z}(0)\rangle$, $\frac{d}{dt}C_{vv}(t) = \langle\delta\ddot{z}(t)\delta\dot{z}(0)\rangle$, and $\int_0^t C_{vv}(t)dt = \langle\delta z(t)\delta\dot{z}(0)\rangle$, and take the Laplace transform of both sides, yielding,

$$-\mathcal{L}\left\{m\frac{d}{dt}C_{vv}(t) + K\int_0^t C_{vv}(t)dt\right\} = \mathcal{L}\left\{\int_0^t M(t-t')C_{vv}(t)dt'\right\} \quad (4.29)$$

We invoke the following properties of the Laplace transform,

$$\begin{aligned} \mathcal{L}\left\{\frac{d}{dt}C_{vv}(t)\right\} &= s\tilde{C}_{vv}(s) - \langle\delta\dot{z}^2\rangle \\ \mathcal{L}\left\{\int_0^t C_{vv}(t)dt\right\} &= \frac{1}{s}\tilde{C}_{vv}(s) \\ \mathcal{L}\left\{\int_0^t M(t-t')C_{vv}(t)dt'\right\} &= \tilde{M}(s)\tilde{C}_{vv}(s) \end{aligned} \quad (4.30)$$

where $\langle \delta \dot{z}^2 \rangle = C_{vv}(0)$. We also substitute $m = k_B T / \langle \delta \dot{z}^2 \rangle$ and $K = k_B T / \langle \delta z^2 \rangle$, and solve for the memory function,

$$\begin{aligned}
\tilde{M}(s) \tilde{C}_{vv}(s) &= -\frac{k_B T}{\langle \delta \dot{z}^2 \rangle} [s \tilde{C}_{vv}(s) - \langle \delta \dot{z}^2 \rangle] - \frac{k_B T}{s \langle \delta z^2 \rangle} \tilde{C}_{vv}(s) \\
\tilde{M}(s) \tilde{C}_{vv}(s) \langle \delta \dot{z}^2 \rangle \langle \delta z^2 \rangle &= -k_B T \left(\langle \delta z^2 \rangle [s \tilde{C}_{vv}(s) - \langle \delta \dot{z}^2 \rangle] + \frac{\langle \delta \dot{z}^2 \rangle}{s} \tilde{C}_{vv}(s) \right) \\
\tilde{M}(s) &= -k_B T \frac{s \langle \delta z^2 \rangle \tilde{C}_{vv}(s) - \langle \delta z^2 \rangle \langle \delta \dot{z}^2 \rangle + \frac{\langle \delta \dot{z}^2 \rangle}{s} \tilde{C}_{vv}(s)}{\langle \delta \dot{z}^2 \rangle \langle \delta z^2 \rangle \tilde{C}_{vv}(s)} \\
\tilde{M}(s) &= -k_B T \frac{\tilde{C}_{vv}(s) [s \langle \delta z^2 \rangle + \langle \delta \dot{z}^2 \rangle / s] - \langle \delta z^2 \rangle \langle \delta \dot{z}^2 \rangle}{\langle \delta \dot{z}^2 \rangle \langle \delta z^2 \rangle \tilde{C}_{vv}(s)}
\end{aligned} \tag{4.31}$$

which is the memory function in Laplace space used for computing the Woolf-Roux diffusivity. In order to derive the memory function of the Hummer diffusivity, we first obtain $\tilde{C}_{vv}(s)$ in terms of $\tilde{C}_{zz}(s)$ by,

$$\begin{aligned}
-\mathcal{L}\left\{C_{vv}(t)\right\} &= \mathcal{L}\left\{\frac{d^2}{dt^2}C_{zz}(t)\right\} \\
&= s\mathcal{L}\left\{\frac{d}{dt}C_{zz}(t)\right\} - \langle \delta \dot{z} \delta z \rangle \\
&= s(s\tilde{C}_{zz}(s) - \langle \delta z^2 \rangle) \\
\tilde{C}_{vv}(s) &= -s^2\tilde{C}_{zz}(s) + s\langle \delta z^2 \rangle
\end{aligned} \tag{4.32}$$

and then substitute this result into eq 4.31. The memory function of the Hummer diffusivity in Laplace space is given by,

$$\begin{aligned}
\tilde{M}(s) &= -k_B T \frac{(-s^2\tilde{C}_{zz}(s) + s\langle \delta z^2 \rangle) [s\langle \delta z^2 \rangle + \langle \delta \dot{z}^2 \rangle / s] - \langle \delta z^2 \rangle \langle \delta \dot{z}^2 \rangle}{\langle \delta \dot{z}^2 \rangle \langle \delta z^2 \rangle (-s^2\tilde{C}_{zz}(s) + s\langle \delta z^2 \rangle)} \\
&= k_B T \frac{\tilde{C}_{zz}(s) [s^2\langle \delta z^2 \rangle + \langle \delta \dot{z}^2 \rangle] - s\langle \delta z^2 \rangle^2}{\langle \delta \dot{z}^2 \rangle \langle \delta z^2 \rangle (\langle \delta z^2 \rangle - s\tilde{C}_{zz}(s))}
\end{aligned} \tag{4.33}$$

CHAPTER 5

SYSTEMATIC SHORT UNBIASED TRAJECTORY SIMULATION WITH GENERALIZED LANGEVIN AUTOMATED KINETIC ENHANCED SAMPLING (GLAKES)

5.1 Introduction

An enhanced sampling method called generalized Langevin automated kinetic enhanced sampling (gLakes), based on a previous approach that involved re-weighting the committor time-correlation function to extract the permeability is presented.¹³⁶ The gLakes method was used to rapidly determine the permeability with numerical GLE simulations, which is shown to converge orders of magnitude faster than the brute force GLE simulations. The numerical GLE permeability, when readily computable, is demonstrated to be a convenient way to evaluate the validity of ISD model in the context of the memory function of the underlying GLE.

5.2 Theoretical developments

5.2.1 Estimating permeability via generalized Langevin automated kinetic enhanced sampling (gLakes)

To efficiently and accurately solve the rate of barrier crossing for the GLE with position-dependent memory and the PMF extracted from the MD simulations, we introduce an enhanced sampling scheme known as generalized Langevin automated kinetic enhanced sampling (gLakes). This approach is based on the equilibrium committor time-correlation function,¹⁸

$$C_{qq}(t) = \frac{1}{2} \langle (q(t) - q(0))^2 \rangle \quad (5.1)$$

where the permeability is proportional to the slope in a long lag time limit,¹³

$$P_{qq} = \frac{1}{\pi(-L/2)} \lim_{t \rightarrow \tau_q} \dot{C}_{qq}(t) \quad (5.2)$$

where τ_q is the Markovian lag time at which the slope becomes constant. The committor time-correlation function can also be calculated by re-weighting conditional averages along z . In a long equilibrium simulation, the conditional averages along z are naturally weighted by the equilibrium probability. In an enhanced sampling scheme it is possible to initiate short unbiased simulations according to a more advantageous distribution if the underlying equilibrium probability distribution is already known.¹³⁶ In terms of the conditional committor-time correlation functions from discretized bins along z , the re-weighted $C_{qq}(t)$ is given by,

$$C_{qq}(t) = \sum_{n=1}^N \frac{\pi_n \Delta z}{w_n} \sum_{i_n=1}^{w_n} c(t; z_{i_n}) \quad (5.3)$$

where z_{i_n} is the i th trajectory with an initial position that falls in bin n , w_n is the total number of trajectories beginning in bin n , π_n is the equilibrium probability of bin n , and $c(t; z_{i_n})$ is the conditional committor time-correlation function. A similar approach based on bin-less WHAM was applied by Chen et al.¹⁵² The conditional committor time-correlation function for a trajectory with $z(0) = z_{i_n}$ is given by,

$$c(t^*; z_{i_n}) = \delta(z_n - z_{i_n}(0)) \frac{1}{n_t} \sum_{k=1}^{n_t} \frac{1}{2} (q(k\Delta t + t^*) - q(k\Delta t))^2 \quad (5.4)$$

where $n_t \Delta t$ is the total length of the trajectory. The choice of w_n can be made to allow $C_{qq}(t)$ to converge with as few trajectories as possible. A second advantage is that the length of each trajectory only needs to be as long as the Markovian lag time τ_q . The optimal distribution of w_n for minimizing the uncertainty in the final $C_{qq}(t)$ is given by Neyman

allocation,¹⁵³

$$w_n = M_{\text{tot}} \times \frac{\sigma_n \pi_n}{\sum_{n=1}^N \sigma_n \pi_n} \quad (5.5)$$

where M_{tot} is the total number of short trajectories across all bins, $M_{\text{tot}} = \sum_{n=1}^N w_n$ and σ_n is the standard deviation of the time-correlation function computed within bin n .

5.3 Implementation details

gLakes simulations

Within the gLakes algorithm, the optimal distribution of initial positions of short unbiased trajectories, w_n , is solved iteratively, starting from a uniform distribution. At each iteration, σ_n is estimated based on the current conditional committor time-correlation functions,

$$\sigma_n^i(t) = \sqrt{\langle c(t; z_n)^2 \rangle_i - \langle c(t; z_n) \rangle_i^2} \quad (5.6)$$

where the mean of the conditional time-correlational function in iteration i is given by,

$$\langle c(t; z_n) \rangle_i = \frac{1}{w_n^i} \sum_{j=1}^{w_n^i} c(t; z_n) \quad (5.7)$$

Because there is a unique value of σ_n^i for each lag time t , it is necessary either to choose a single lag time or to take an average over time. For simplicity, we took the standard deviation in each bin as the mean over lag time,

$$\bar{\sigma}_n^i = \frac{1}{n_t} \sum_{k=1}^{n_t} \sigma_n^i(k\Delta t) \quad (5.8)$$

Finally, the running sum of the samples in each bin is updated according to,

$$w_n^{i+1} = w_n^i + M^{i+1} \times \frac{\bar{\sigma}_n^i \pi_n}{\sum_{n=1}^N \bar{\sigma}_n^i \pi_n} \quad (5.9)$$

where M^{i+1} is the number of new trajectories to be run in iteration $i + 1$. In practice, randomness is introduced into w_n at each iteration. First, the finely spaced $[w_n^{i+1} - w_n^i]$ computed with eq 5.9 is symmetrized about $z = 0$ and smoothed by a rolling mean with a window of 11 bins. The initial z positions of each short unbiased trajectory are then obtained by generating M^{i+1} uniform random numbers (between 0 and 1) and finding the corresponding set of z_n within the cumulative distribution function of the smoothed $[w_n^{i+1} - w_n^i]$.

The additional capability of gLakes to decrease the computational cost of calculating the permeability is the automated updates of the estimated τ_q . At the end of each iteration, an analytical function is fit by least-squares to the current estimate of $C_{qq}(t)$. The analytical function consists of a decaying exponential term and a linear term,

$$C_{qq}^{fit}(t) = -a_0 \exp[-a_1 t] + a_2 t + a_3 \quad (5.10)$$

The new estimate of τ_q^i is calculated based on the lag time at which the derivative of the exponential function has decayed to a negligible fraction of the derivative of the linear term, such that,

$$\begin{aligned} a_2 &\approx C a_0 a_1 \exp[-a_1 \tau_q^{i+1}] \\ \tau_q^{i+1} &\approx -\frac{1}{a_1} \ln \left[\frac{a_2}{C a_0 a_1} \right] \end{aligned} \quad (5.11)$$

where C is a positive constant for softening chosen to be much larger than 1. In practice, at each iteration, τ_q is moving according to a “slide” constant, S_c , such that its value does not

decrease too sharply, $\tau_q^{i+1} = \tau_q^i - (\tau_q^i - \tau_q^{i+1})/S_C$.

Following the gLakes procedure, a total of 91 iterations of 1000 short-unbiased trajectories each were run in order to optimize the re-weighted committor time-correlation function (eq 5.3). The initial length of each short-unbiased trajectory (τ_q) was set to 50 ps, and allowed to decrease automatically at each iteration according to eq 5.11, where the softening constant C was set to 100. The minimum value of τ_q was constrained to 10 ps. The slide constant, S_C , was set to 4, which is shown to be a satisfactory guess.

The committor probability function itself was constructed in approximate form based on the PMF,¹⁸

$$q(z) \approx \frac{\int_{-L/2}^z dz' e^{W(z')/k_B T}}{\int_{-L/2}^{L/2} dz' e^{W(z')/k_B T}} \quad (5.12)$$

For maximum efficiency, the approximate committor was fit to a hyperbolic tangent function of the form,

$$q(z) \approx \frac{1}{2} (\tanh(m_0 z) + 1) \quad (5.13)$$

where m_0 was determined to be $0.309 \text{ \AA}^{-1}$ via least-squares regression.

For the discrete z -axis, the bin width was set to $0.1 \text{ \AA}$. For the time-axis, the spacing is 0.1 ps . An important note for the reliable convergence of the gLakes method, which makes use of short unbiased trajectories initiated out of equilibrium, is appropriate initialization of the auxiliary Markovian variables, $S_i(0)$, and velocity, $\dot{z}(0)$, of eq 4.20. In practice, each of these were initialized to Gaussian random numbers with mean 0 and variance corresponding to $k_B T K_i(z(0))$ and $k_B T/m$ for $S_i(0)$ and $\dot{z}(0)$, respectively.

5.4 Results

5.4.1 *Extraction of the permeability coefficient with gLakes*

In the previous section, we have shown that the permeability of the GLE with position-dependent memory can be extracted from brute force numerical integration by counting membrane crossing events. Here, gLakes is shown to be a way to compute the permeability of the GLE without resorting to brute force simulations, while relaxing the requirements of a position-independent memory and parabolic PMF imposed by Grote-Hynes theory. This is necessary because there is no closed-form mathematical expression to perform this task without approximation. The result can be compared with the ISD permeability to assess the significance of memory effects on the process and system that is being studied. The workflow proceeds as follows,

1. Run umbrella sampling MD simulations, using the z -coordinate as the collective-variable.
2. Determine the PMF and the time-correlation functions needed for computing $D(z)$.
3. Calculate the Woolf-Roux and Hummer $D(z)$.
4. Construct the modified loss function (eq 4.10) and parameterize the GLE via the MLP deep learning procedure.
5. Construct the GLE coarse-grained model using the position-dependent memory function and the PMF obtained from umbrella sampling.
6. Run the gLakes algorithm to efficiently extract permeability.

The gLakes approach relies on iterative sets of short unbiased simulations propagated by the numerical GLE integration scheme. At each iteration, the cumulative set of unbiased trajectories is used to estimate the total committor time-correlation function based on the

re-weighting of eq 5.3. The cycle is continued by initiating the next set of short unbiased simulations according to the proportions prescribed by Neyman allocation (eq 5.5), which prioritizes regions of z with a large product of standard deviation in the committor-correlation function and the equilibrium probability known from the PMF.

The cumulative distribution of initial positions, w_n , at each of the 91 iterations of 1000 short unbiased trajectories each is shown in Figure 5.1A. Similarly, the convergence of $C_{qq}(t)$ computed from the cumulative set of simulations at each iteration is shown in Figure 5.1B. Using the counting permeability from the brute force numerical GLE simulations as a reference, we show that the permeability extracted from each iteration of $C_{qq}(t)$ has largely converged after 25 iterations in Figure 5.1C. In iteration 25, the permeability was estimated to be 0.0100 cm/s, which corresponds to a 0.4 % error with respect to the brute force counting permeability. The total length of each short unbiased trajectory at each iteration, τ_q^i , was minimized iteratively to further improve computational efficiency. The initial value of τ_q was set to 50 ps, and it was automatically reduced to 29 ps by the 5th iteration. Therefore, the total simulation time to obtain an estimate of the permeability in close agreement with the brute force result was 805 ns.

We attribute the efficiency of the gLakes algorithm to the implementation of Neyman allocation via iteratively updating w_n based on the current estimate of the standard deviation in each conditional committor time-correlation function and known equilibrium probability of each bin. The optimized w_n after 91 iterations reveals the complex interplay between the equilibrium probability distribution and the standard deviation within each local contribution to the re-weighted committor time-correlation function. The rapid convergence within 805 ns to a reasonable and stable estimate of the permeability can be contextualized by considering how much brute force sampling would be required to achieve a similar estimate. Considering a permeability of approximately 0.01 cm/s, 678 μ s of sampling on average would be needed to observe just 20 crossing events.

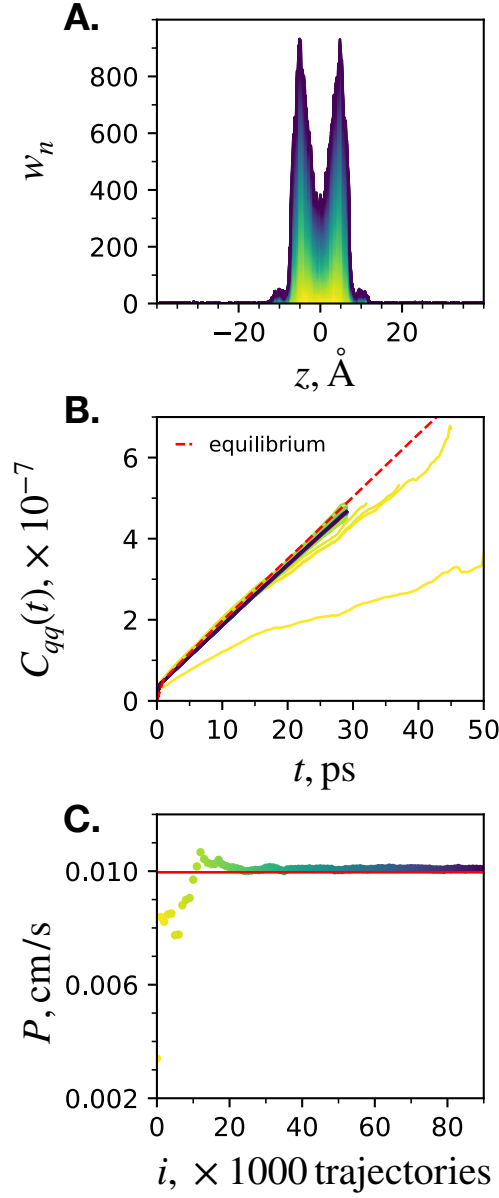


Figure 5.1: Results of the gLakes simulations for the coarse-grained water-membrane system. A total of 91 iterations of 1000 short trajectories were run. **A.** The cumulative distributions of initial positions (w_n) for each iteration. **B.** The committor time-correlation function calculated at each iteration, along with the result from the long equilibrium simulations. The time-correlation functions become shorter as τ_q is updated by the algorithm. **C.** The permeability based on the slope of the committor-time correlation function at each iteration. Also shown is the counting permeability of the long equilibrium simulations.

5.5 Conclusion

In principle, memory effects can be assessed with Grote-Hynes theory, assuming that the memory function is constant with respect to position, and the PMF can be approximated by an inverted parabola. In the more academic case in which the memory function is a positive, decaying exponential and the mass is taken to the limit of zero, the permeability can even be computed analytically.¹³ Because there is not a closed form expression to solve the GLE with an arbitrary PMF and position-dependent memory, it was necessary to numerically integrate the GLE. The GLE was numerically integrated efficiently with Markovian embedding, which was possible due to the assumed form of the memory function.¹⁵ To solve the GLE for the permeability as efficiently as possible, the gLakes algorithm was developed. The gLakes approach, which is based on strategically initiating short unbiased trajectories along the reaction coordinate and computing a re-weighted committor time-correlation function, revealed that the GLE permeability and the overdamped ISD permeability are in good agreement. This result indicates that based on the memory function extracted from the umbrella sampling MD simulations with the MLP and the PMF from equilibrium MD simulations, there is most likely not a significant impact of memory effects and therefore the ISD model is valid.

CHAPTER 6

HOW IS MEMBRANE PERMEATION OF SMALL IONIZABLE MOLECULES AFFECTED BY PROTONATION KINETICS?

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<https://pubs.acs.org/doi/full/10.1021/acs.jpcb.3c06765>

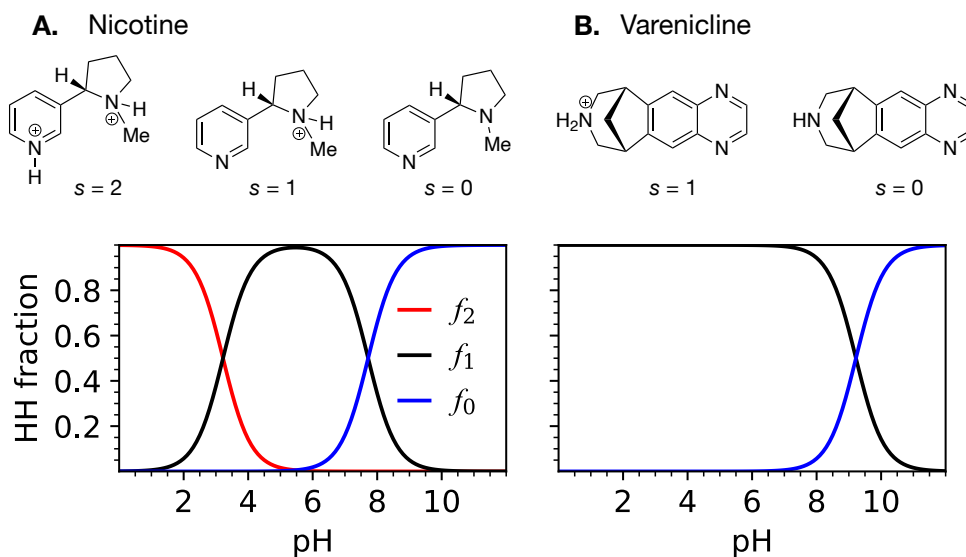
ABSTRACT – According to the pH-partition hypothesis, the aqueous solution adjacent to a membrane is a mixture of the ionization states of the permeating molecule at fixed Henderson–Hasselbalch concentrations, such that each state passes through the membrane in parallel with its own specific permeability. An alternative view, based on the assumption that the rate of switching ionization states is instantaneous, represents the permeation of ionizable molecules via an effective Boltzmann-weighted average potential (BWAP). Such an assumption is used in constant-pH molecular dynamics simulations. The inhomogeneous solubility-diffusion framework can be used to compute the pH-dependent membrane permeability for each of these two limiting treatments. With biased WTM-eABF molecular dynamics simulations, we computed the potential of mean force and diffusivity of each ionization state of two weakly basic small molecules: nicotine, an addictive drug, and varenicline, a therapeutic for treating nicotine addiction. At pH = 7, the BWAP effective permeability is greater than that determined by pH-partitioning by a factor of 2.5 for nicotine and 5 for varenicline. To assess the importance of ionization kinetics, we present a Smoluchowski master equation that includes explicitly the protonation and deprotonation processes coupled with the diffusive motion across the membrane. At pH = 7, the increase in permeability due to the explicit ionization kinetics is negligible for both nicotine and varenicline. This

finding is reaffirmed by combined Brownian dynamics and Markov state model simulations for estimating the permeability of nicotine while allowing changes in ionization state. We conclude that for these molecules the pH-partition hypothesis correctly captures the physics of the permeation process. The small free energy barriers for the permeation of nicotine and varenicline in their deprotonated neutral forms play a crucial role in establishing the validity of the pH-partitioning mechanism. Essentially, BWAP fails because ionization kinetics are too slow on the timescale of membrane crossing to affect the permeation of small ionizable molecules such as nicotine and varenicline. For the singly protonated state of nicotine, the computational results agree well with experimental measurements ($P_1 = 1.29 \times 10^{-7}$ cm/s), but the agreement for neutral ($P_0 = 6.12$ cm/s) and doubly protonated nicotine ($P_2 = 3.70 \times 10^{-13}$ cm/s) is slightly worse, likely due to factors associated with the aqueous boundary layer (neutral form) or leaks through paracellular pathways (doubly protonated form).

6.1 Introduction

Calculating membrane permeability to small ionizable molecules from first principles is an engaging problem of biophysical research from both theoretical and practical perspectives.^{19;32} It is also of great interest to drug development,^{20;19;33} as many drugs administered orally have different ionization states in water and membranes, and are active against their dedicated target in an ionized form.^{154;25} Nicotine and varenicline provide interesting examples of permeation of small ionizable molecules through biological membranes. Nicotine is an alkaloid¹⁵⁵ with two protonation sites, a pyrrolidine nitrogen with a pK_a of 7.72, and a pyridine nitrogen with a pK_a of 3.22, as determined by titration at 313 K.¹⁵⁶ Varenicline is also a small ionizable molecule, with similar chemical properties to nicotine.^{157;158;159} It has one protonation site, an aliphatic amine with $pK_a = 9.22$, determined by titration at 298 K.¹⁶⁰ The chemical structures and the fractions of each protonation state of nicotine and

varenicline based on the classic Henderson–Hasselbalch equation are shown in Figure 6.1. The relative permeability of these molecules plays an important role in the effectiveness



centering

Figure 6.1: Chemical structures and Henderson–Hasselbalch fractions of each protonation state (f_0 = neutral, f_1 = singly protonated, f_2 = doubly protonated) based on aqueous pK_a over a range of pH for **A.** nicotine ($pK_a = 3.22$ for $s = 2$, and $pK_a = 7.72$ for $s = 1$) and **B.** varenicline ($pK_a = 9.22$ for $s = 1$)

of the drug varenicline (commercially known as Champix or Chantix) for treating nicotine addiction. It has been shown that both molecules partition into low-pH vesicles containing nicotinic acetylcholine receptors (nAChRs) in rat neurons.^{161;162;163} Nicotine remains in the brain for only 1–2 hours, whereas varenicline can apparently stay for as long as 4–5 days.¹⁶² Green et al.¹⁶² conjectured that varenicline becomes trapped partly due to its higher binding affinity for the nAChR receptor.^{159;164} They also note that the wide kinetic disparity between nicotine and varenicline may be partially explained by the so-called pH-partition hypothesis, which assumes that the effective permeability is controlled by the equilibrium Henderson–Hasselbalch fraction of each ionization state present in bulk solution.¹⁶² Because varenicline has a larger pK_a than nicotine, the equilibrium concentration of its deprotonated (neutral) form is effectively smaller at low pH (Figure 6.1), yielding a smaller permeability and a slower release from the brain. The pH-partition mechanism is illustrated by the

application of filling vesicles with small ionizable molecules using pH gradients.^{25;165} The mechanism is also consistent with the absorption of nicotine from gum through cheek cells, which is decreased after drinking acidic coffee.^{166;167} Nicotine and varenicline can be used to illustrate the impact of pH and pK_a on the effective permeability of ionizable molecules.

In the context of the present work, we refer to the simulation-derived permeability of a single ionization state as the *specific permeability*, and the simulation-derived pH-dependent permeability of the combined states as the *effective permeability*. These are distinct from experimental values, which are presently referred to as *apparent specific permeability* for a single ionization state, and *apparent permeability* for the pH-dependent value.¹⁶⁸ A popular theoretical framework to compute the specific permeability coefficient of a molecule is provided by the classic Kramers-Smoluchowski treatment of the steady state flux across a free energy barrier.^{7;34} Within this framework, the permeability P is expressed as,

$$\frac{1}{P} = \int_{-L/2}^{L/2} dz \frac{e^{+[W(z)-W(-L/2)]/k_B T}}{D(z)} \quad (6.1)$$

where L is the membrane thickness, and $D(z)$ and $W(z)$ are the position-dependent diffusivity and the potential of mean force (PMF) of the molecule of interest along the membrane normal, z , respectively. Both $D(z)$ and $W(z)$ can be calculated from MD simulations. The membrane thickness L in eq 6.1 should be chosen such that the PMF, $W(z)$, is equal to a constant when $|z| > L/2$ in the bulk region (the constant can be set to zero for convenience). This theoretical framework, commonly referred to as “inhomogeneous solubility-diffusion” (ISD) since the pioneering work of Diamond and Katz,⁸ has been applied to a variety of molecules.^{6;35;36;37;38;39;40} The simplest form of the ISD framework needs to be extended in the case of ionizable molecules. In the pH-partition mechanism mentioned above, it is assumed that the interconversion protonation/deprotonation rates have a negligible impact on the effective permeability. The bulk solution is treated as an equilibrium mixture of each ionization state at fixed concentrations based on the Henderson–Hasselbalch equa-

tion.^{169;25;168;166} On the basis of this physical assumption, one can determine the specific permeability for each ionization state s within the ISD framework via eq 6.1 using the corresponding PMF and diffusivity, $W_s(z)$ and $D_s(z)$. The effective permeability of the ionizable molecule is then,

$$P(\text{pH}) = \sum_s f_s(\text{pH}) P_s \quad (6.2)$$

where $f_s(\text{pH})$ is the Henderson–Hasselbalch fraction of ionization state s , and P_s is the specific permeability of state s .¹⁷⁰ The pH-partition mechanism is supported experimentally by measurements of cell permeability to small ionizable molecules at a range of pH conditions.¹⁶⁸ From this type of study, the apparent specific permeability of each protonation state can be derived by solving eq 6.2 for all P_s , where $P(\text{pH})$ has been measured for at least as many pH values as there are s values. The pH-partition hypothesis has also been supported by fluorescence experiments with large unilamellar vesicles (LUV).^{118;23;171} An example is the study of propranolol permeability by Eyer et al.,²³ which illustrates the separation of timescales between ionization kinetics and permeation of the neutral species. The rate-limiting effect of ionization kinetics on LUV permeation measurements was pointed out by Sezer et al.,²⁵ who inferred the rate of protonation of propranolol from the fluorescence experiment results²³ to be around $10^{10.67} \text{ M}^{-1} \cdot \text{s}^{-1}$, in good agreement with the previously established experimental range for small molecules of 10^{10} to $10^{11} \text{ M}^{-1} \cdot \text{s}^{-1}$.¹⁷²

A radically different physical picture from pH-partitioning is obtained if it is assumed that the interconversion protonation/deprotonation rates are much faster than the spatial diffusion of the permeant. In this case, the permeating molecule is expected to diffuse along an effective pH-dependent potential corresponding to a Boltzmann-weighted average of the individual $W_s(z)$ of each ionization state s .^{173;27} The effective average potential can then be used within the ISD framework to calculate the effective permeability via eq 6.1. This is the approach used by Oung et al.¹⁷³ to compute membrane permeability to cocaine. Assuming fast interconversion rates on the timescale of an MD simulation is

formally equivalent to the results obtained by employing constant-pH MD (CpHMD) simulations,^{174;175;176;177;178;179;180;181} which impose equilibrium partition among all of the ionization states for every configuration of a system.²⁷ This is the approach used by Yue et al.²⁷ to compute the effective permeability of propranolol. This Boltzmann-weighted average potential (BWAP) treatment assumes virtually instantaneous changes in ionization state, presenting a very different physical picture from the stable mixtures at fixed concentration assumed in the pH-partitioning model. Despite these differences, both treatments rely on statistical mechanical equilibrium, although at different stages. Ultimately, pH-partitioning and the BWAP are both approximate treatments, and assessing their relative validity for computing the effective permeability of ionizable molecules is important.

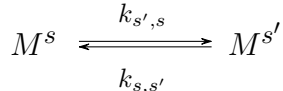
In the present work, we use the enhanced-sampling algorithm well-tempered metadynamics with extended adaptive biasing force (WTM-eABF)^{35;36} to compute the specific permeability of each of the ionization states of two small ionizable molecules, nicotine and varenicline. This approach has been previously applied to two protonation states of ibuprofen.⁴⁰ Enhanced sampling is employed first for computing the PMF underlying permeation along the membrane normal, and then for determining the position-dependent diffusivity by running trajectories on the flattened free energy surface. The PMF and diffusivity profile are then combined to compute the effective permeability within the ISD framework,⁸ as per eq 6.1. We also introduce a Smoluchowski master equation that includes explicitly the experimental rates of protonation and deprotonation, along with the diffusive motion across the membrane. This framework is helpful to clarify how the kinetics of the protonation/deprotonation events should be treated in conjunction with the spatial diffusion process, as well as to assess the relative validity of the pH-partitioning and the BWAP treatments. Moreover, an analysis based on the transition path theory (TPT) framework^{67;17;68;69;70;18} is exploited to quantitatively estimate the impact of ionization kinetics on the permeation process and to examine the circumstances in which the pH-partitioning and the BWAP

treatments yield similar or dissimilar results. The results of the transition path theory (TPT) analysis are supported by combined Brownian dynamics and Markov state model simulations⁹ to estimate the permeability of nicotine while allowing changes in ionization state. Finally, a special effort is made to reconcile the computed permeability coefficients with experimental data, explicitly considering the effects of ionization kinetics, the aqueous boundary layer, and the limitations of our simulations.

6.2 Theoretical Developments

6.2.1 Equilibrium and kinetics of ionizable states

We begin with the classic treatment of an ionizable molecule, M , with multiple ionization states, s , in bulk solution,



where the transition rates $k_{s',s}$ indicate a transition from state s to state s' . The master equation describing the discrete ionization process is,

$$\frac{d[M^s]}{dt} = \sum_{s'} k_{s,s'} [M^{s'}] \quad (6.3)$$

The diagonal term $k_{s,s} = -\sum_{s' \neq s} k_{s',s}$ corresponds to the total rate of probability leaking out of state s . State $s = 0$ corresponds to the deprotonated molecule. By construction, s and s' must be greater than 0, and smaller than some maximum $s_{\max}$. Because the ionization process typically occurs one proton at a time, the possible states s and s' are constrained by $s' = s \pm 1$. The equilibrium Henderson–Hasselbalch fractions of the ionization states follow from,

$$\frac{f_{s+1}}{f_s} = \frac{k_{s+1,s}}{k_{s,s+1}} = 10^{(\text{p}K_a(s+1) - \text{pH})} \quad (6.4)$$

with $\sum_s f_s = 1$. The transition rate for protonation (often called “ k_{on} ”) can be expressed as $k_{s+1,s} = k_0 \times 10^{-\text{pH}}$, reflecting a bimolecular reaction that depends on the proton concentration, $[\text{H}^+]$. In solution, the intrinsic rate k_0 is on the order of $10^{10} \text{ M}^{-1} \cdot \text{s}^{-1}$,¹⁷² yielding a protonation rate $k_{s+1,s}$ that is on the order of 10^3 s^{-1} at neutral pH. The deprotonation rate $k_{s,s+1}$ (often called “ k_{off} ”) reflects a unimolecular reaction that is essentially prescribed by the Henderson–Hasselbalch equilibrium according to eq 6.4, with $k_{s,s+1} = k_0 \times 10^{-\text{p}K_{\text{a}}(s+1)}$. To extend these classic ideas to the problem of membrane permeation, the intrinsic rate k_0 should depend on the water density and, thus, be a function of z . Conceivably, changes in protonation state would occur extremely slowly or not at all if there are no nearby water molecules in the membrane interior. Consequently, the transition rates $k_{s+1,s}$ and $k_{s,s+1}$ could be reduced significantly in the membrane region. Nevertheless, the Henderson–Hasselbalch equilibrium governed by eq 6.4 is not affected by $k_0(z)$. The $\text{p}K_{\text{a}}$ of state s , however, depends on the z position of the molecule along the membrane normal, according to the difference in the PMFs of adjacent states,

$$\text{p}K_{\text{a}}(z, s) = \text{p}K_{\text{a}}(\infty, s) - \frac{1}{\log[10]k_{\text{B}}T} (W_s(z) - W_{s-1}(z)) \quad (6.5)$$

The position dependence of the deprotonation rate, $k_{s,s+1}(z) = k_0 \times 10^{-\text{p}K_{\text{a}}(z,s+1)}$, is therefore contained in eq 6.5.

6.2.2 Spatial diffusion and discrete ionization

We consider a membrane separating two solutions. The configuration of the system for a permeating molecule is determined by its position z and ionization state s . Combining the discrete ionization process with the spatial diffusion of the molecule along the z -axis across

the membrane yields the Smoluchowski master equation (SME),

$$\frac{\partial p_s(z, t)}{\partial t} = \frac{\partial}{\partial z} \left(\frac{D_s(z)}{k_B T} \frac{\partial W_s(z)}{\partial z} p_s(z, t) + D_s(z) \frac{\partial p_s(z, t)}{\partial z} \right) + \sum_{s'} k_{s, s'}(z) p_{s'}(z, t) \quad (6.6)$$

where $W_s(z)$ is the PMF of the ionization state s , and $p_s(z, t)$ is the normalized time-dependent probability density, with $\sum_s \int_{-L/2}^{L/2} p_s(z, t) dz = 1$. The SME (eq 6.6) is inspired by previous work by Berezhkovskii et al.¹⁸² The membrane thickness L should be chosen such that $W_s(z)$ reaches a constant value at $z = \pm L/2$, indicating the beginning of the bulk solvent region. For the sake of convenience, all of the PMFs are then set to obey the condition $W_s(z) = 0$ at $z = \pm L/2$. We define the left side of the membrane ($z \leq -L/2$) as the reactant state and the right side ($z \geq L/2$) as the product state, and the net flux of permeant molecules, J , is expected to go from left to right. The normalized equilibrium probability density of state s in the membrane region, i.e., $-L/2 < z < L/2$, is

$$\pi_s(z) = \frac{f_s e^{-W_s(z)/k_B T}}{\sum_s f_s \int_{-L/2}^{L/2} dz e^{-W_s(z)/k_B T}} \quad (6.7)$$

with $\sum_s \int_{-L/2}^{L/2} \pi_s(z) dz = 1$. The z -dependent discrete state transition rates obey detailed balance, namely $k_{s', s}(z) \pi_s(z) = k_{s, s'}(z) \pi_{s'}(z)$, and the local probability density at the boundary,

$$f_s = \frac{\pi_s(-L/2)}{\sum_{s'} \pi_{s'}(-L/2)} \quad (6.8)$$

recovers the bulk Henderson–Hasselbalch population fraction for each state. To determine the effective permeability coefficient, P , a steady state flux J is created by imposing the equilibrium probability at $z = -L/2$, and an absorbing boundary at $z = L/2$, according to $P = J (\sum_s p_s(-L/2))^{-1}$. J is the total flux due to all ionization states. Two limits can be delineated: when the ionization process is much slower than the spatial diffusion

(“slow interconversion rates”), or when the ionization process is much faster than the spatial diffusion (“fast interconversion rates”). These two limits correspond to the pH-partitioning and the Bwap approximate treatments, respectively.

Slow interconversion rates: pH-partitioning

When the rates of the protonation/deprotonation transitions are much slower than the diffusive dynamics, permeation proceeds as an equilibrium superposition of the ionization states. The steady state flux J_s of each ionization state, s , can then be determined separately,

$$J_s = -\frac{D_s(z)}{k_B T} \frac{\partial W_s(z)}{\partial z} p_s(z) - D_s(z) \frac{\partial p_s(z)}{\partial z} \quad (6.9)$$

where $p_s(z)$ is the normalized steady-state probability density, yielding

$$\begin{aligned} J_s &= p_s(-L/2) \left(\int_{-L/2}^{L/2} dz \frac{e^{+W_s(z)/k_B T}}{D_s(z)} \right)^{-1} \\ &= p_s(-L/2) P_s \end{aligned} \quad (6.10)$$

with $p_s(-L/2) = \pi_s(-L/2)$. The total steady-state flux summed over all states s is

$$\begin{aligned} J &= \sum_s J_s \\ &= \sum_s \pi_s(-L/2) P_s \end{aligned} \quad (6.11)$$

The phenomenological constitutive relation, $J = C(-L/2) P$, defines the permeability coefficient P by expressing the total flux J (left to right) in terms of the permeant concentration C in the bulk solution (on the left side of the membrane). By correspondence, the Kramers-Smoluchowski treatment relates J to the total probability $\sum_s \pi_s(-L/2)$ at the left boundary

of the system,

$$J = \left(\sum_s \pi_s(-L/2) \right) P \quad (6.12)$$

Combining eq 6.11 with eq 6.12 yields,

$$\begin{aligned} P^{\text{pH-partition}} &= \sum_s \frac{\pi_s(-L/2)}{\sum_{s'} \pi_{s'}(-L/2)} P_s \\ &= \sum_s f_s P_s \end{aligned} \quad (6.13)$$

where P_s is the specific permeability of state s , and P^{pH} is the effective permeability. This is the equilibrium pH-partition treatment.

Fast interconversion rates: Boltzmann-weighted average potential

When the rates of the protonation/deprotonation transitions are much faster than the spatial diffusion, the ionization state of the diffusing molecule changes many times before its position changes appreciably. Because of the fast protonation/deprotonation process, a local Boltzmann equilibrium between the populations of each discrete state, s , is established at each z ,

$$\frac{p_s(z)}{p_{s'}(z)} = \frac{f_s e^{-W_s(z)/k_B T}}{f_{s'} e^{-W_{s'}(z)/k_B T}} \quad (6.14)$$

The probability of each ionization state s reflects the local Henderson–Hasselbalch equilibrium among the discrete states at all z . In this limit, one obtains the reduced Smoluchowski equation in terms of the total distribution (summed over all states s), $p(z) = \sum_s p_s(z)$,¹⁸²

$$\frac{\partial p}{\partial t} = \frac{\partial}{\partial z} \left(\frac{D_m}{k_B T} \frac{\partial W_m}{\partial z} p + D_m \frac{\partial p}{\partial z} \right) \quad (6.15)$$

with the effective pH-dependent Boltzmann-weighted average potential (BWAP), $W_m(z)$, defined as,

$$e^{-W_m(z)/k_B T} = \sum_s f_s e^{-W_s(z)/k_B T} \quad (6.16)$$

and the effective diffusion coefficient defined as,

$$D_m(z) = \left(\sum_s \frac{1}{D_s(z)} \frac{f_s e^{-W_s(z)/k_B T}}{\sum_{s'} f_{s'} e^{-W_{s'}(z)/k_B T}} \right)^{-1} \quad (6.17)$$

The expression for the effective diffusivity corresponds to an average of the friction coefficients of each ionization state. The derivation of eq 6.15 follows a classic statistical mechanical treatment in which fast variables are integrated out to obtain the effective reduced dynamics.¹⁸³ Assuming steady-state conditions, the total flux is,

$$\begin{aligned} J &= -\frac{D_m(z)}{k_B T} \frac{\partial W_m(z)}{\partial z} p(z) - D_m(z) \frac{\partial p(z)}{\partial z} \\ &= p(-L/2) \left(\int_{-L/2}^{L/2} dz \frac{e^{+W_m(z)/k_B T}}{D_m(z)} \right)^{-1} \end{aligned} \quad (6.18)$$

and the effective permeability coefficient is

$$P^{\text{BWAP}} = \left(\int_{-L/2}^{L/2} dz \frac{e^{+W_m(z)/k_B T}}{D_m(z)} \right)^{-1} \quad (6.19)$$

We call this development the Boltzmann-weighted average potential (BWAP) treatment.^{173;27}

6.2.3 TPT formulation of the net reactive flux

Both pH-partition and the BWAP are approximate treatments. To better understand the influence of ionization kinetics on the rate of permeation, we expand these formulations based on the Smoluchowski master equation in the context of transition path theory (TPT).^{76;67;17;68;69;70;18;184} We begin by defining the forward propagation step of the probability density $p_s(z, t)$ from the time t to the time $t + \tau$,⁷⁰

$$p_s(z', t + \tau) = \sum_s \int dz \mathcal{P}_\tau(z', s' | z, s) p_s(z, t) \quad (6.20)$$

where $\mathcal{P}_\tau(z', s' | z, s)$ is the Markovian propagator of the system for the continuous diffusive step $z \rightarrow z'$ and the discrete hopping step $s \rightarrow s'$, consistent with eq 6.6. The propagator obeys microscopic detailed balance, $\mathcal{P}_\tau(z, s | z', s') \pi_{s'}(z') = \mathcal{P}_\tau(z', s' | z, s) \pi_s(z)$. A useful approach to formulate TPT considers the net unidirectional reactive flux from a reactant to a product state under non-equilibrium steady-state conditions.⁶⁷ Under non-equilibrium steady-state conditions, there is a net unidirectional flux of permeating molecules from the left to the right side of the membrane, and the probability of the system must satisfy,

$$p_{s'}^{\text{ss}}(z') = \int dz \mathcal{P}_\tau(z', s' | z, s) p_s^{\text{ss}}(z) \quad (6.21)$$

with $p_s^{\text{ss}}(z) = \pi_s(z)$ if $z \leq -L/2$ and $p_s^{\text{ss}}(z) = 0$ if $z \geq L/2$. It can be verified by direct substitution into eq D.2 that the steady-state density can be expressed as, $p_s^{\text{ss}}(z) = \pi_s(z) (1 - q_s(z))$, where $\pi_s(z)$ is the equilibrium distribution and $q_s(z)$ is the committor.⁶⁷ The committor $q_s(z)$, a key ingredient of TPT, is the probability that the system will first go to the product state (right side of the membrane) before going to the reactant state (left side of the membrane) when starting from position z and ionization state s .^{76;67;17;68;69;70;18} When there are rate transitions between two long-lived metastable states such as permeation across a membrane, the committor is close to the eigenfunction corresponding to the slowest

relaxation time of the system.^{185;18} Using this ansatz for $p_s^{\text{ss}}(z)$, the steady-state flux from the left to the right side of the membrane can be expressed as,^{67;18}

$$J = \frac{1}{2\tau} \sum_{s'} \int_{-L/2}^{L/2} dz' \sum_s \int_{-L/2}^{L/2} dz \left(q_{s'}(z') - q_s(z) \right)^2 \mathcal{P}_\tau(z', s' | z, s) \pi_s(z) \quad (6.22)$$

A derivation of eq 6.22 is contained in Appendix D. For Markovian dynamics in the limit of $\tau \rightarrow 0^+$, the net flux from eq 6.22 can be written as $J = J^{\text{diff}} + J^{\text{trans}}$, where,¹⁸

$$J^{\text{diff}} = \sum_s \int_{-L/2}^{L/2} dz \left(\frac{\partial q_s(z)}{\partial z} \right)^2 D_s(z) \pi_s(z) \quad (6.23)$$

and

$$J^{\text{trans}} = \frac{1}{2} \sum_{s,s'} \int_{-L/2}^{L/2} dz \left(q_{s'}(z) - q_s(z) \right)^2 k_{s',s}(z) \pi_s(z) \quad (6.24)$$

The term J^{diff} corresponds to the familiar TPT contribution from a diffusive motion along z ,⁶⁸ while the term J^{trans} corresponds to the contribution from the discrete transitions between different ionization states.¹⁸ If the transition rates $k_{s',s}(z)$ are set to zero, the committor is exactly given by,^{76;18}

$$q_s^{\text{diff}}(z) = \frac{\int_{-L/2}^z dz' e^{W_s(z')/k_{\text{B}}T} / D_s(z')}{\int_{-L/2}^{L/2} dz' e^{W_s(z')/k_{\text{B}}T} / D_s(z')} \quad (6.25)$$

and $J = J^{\text{diff}}$. Using $q_s^{\text{diff}}(z)$ from eq 6.25, it is easy to show that the reactive flux from eq 6.23 recovers the result from the pH-partition plus ISD framework (eq 6.10 and eq 6.13). The committor $q_s^{\text{diff}}(z)$ for the purely diffusive process with no discrete transitions ($k_{s',s}(z) = 0$) can be used as a reference in a perturbative approach to approximate the effect of the discrete

transitions,

$$J^{\text{trans}} \approx \frac{1}{2} \sum_{s,s'} \int_{-L/2}^{L/2} dz \left(q_{s'}^{\text{diff}}(z) - q_s^{\text{diff}}(z) \right)^2 k_{s',s}(z) \pi_s(z) \quad (6.26)$$

This strategy, which should be valid as long as $k_{s',s}(z)$ is small, is analogous to the familiar Rayleigh-Schrödinger perturbation theory of quantum mechanics, which is built upon an expansion from unperturbed eigenfunctions of a reference system.

6.3 Simulations and Methods

6.3.1 WTM-eABF computations

Membrane systems

Computation of membrane permeability to small molecules using MD simulations is relatively well-established.^{186;38;27;37;32;187;40;188;189} Each 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) membrane system consisted of 106 lipids, 10671 water molecules, and the permeant in a single ionization state: neutral nicotine ($s = 0$), singly protonated nicotine ($s = 1$), doubly protonated nicotine ($s = 2$), neutral varenicline ($s = 0$), and singly protonated varenicline ($s = 1$). Each ionization state of nicotine and varenicline was parameterized with CGenFF v2.5.1^{190;191;192} through CHARMM-GUI.^{105;106;193;109} The hydrated lipid bilayers were built with CHARMM-GUI^{105;106} and subsequently equilibrated. Each molecule was added to the system by means of an alchemical transformation. The initial configurations for each sampling window were generated by steered MD (SMD) in NAMD 2.14 with the colvars module.^{194;195} The force fields for the three states of nicotine were validated based on the planarity of the pyridine ring, using the molecular planarity parameter (not shown).¹⁹⁶ The CHARMM36 force field¹⁰¹ was used for POPC¹⁰³ with TIP3P water.¹⁰⁸ The (x, y) -dimensions (where z is the membrane normal) of the membrane plane were approximately

$59 \times 59 \text{ \AA}^2$ for all systems, which is considered a small to intermediate sized patch.¹

MD protocol

For the standard MD protocol in NAMD 2.14,¹⁹⁴ the temperature was set to 308 K with the Langevin thermostat with a damping coefficient of 1 ps^{-1} . This value is not expected to affect the dynamics significantly. The pressure was set to 1 atm with the Langevin piston with a period of 400 fs and decay of 300 fs. Long-range electrostatic interactions were handled by PME with a $1.2\text{-\AA}$ grid spacing. Short-range non-bonded interactions were handled with a smooth $12\text{-\AA}$ cutoff, and force switching for van der Waals interactions. The equations of motion were integrated with the r-RESPA multiple time step integrator and an effective time step of 2 fs. As the simulations progressed, the configurations were wrapped with pbctools as needed, and visualized using VMD.¹⁹⁷

PMF calculations

In order to compute the permeability of the five small molecules (nicotine and two additional ionization states, and varenicline and one additional ionization state), we applied the enhanced sampling algorithm WTM-eABF,^{36;198} which is built upon well-tempered metadynamics (WTM),^{199;200;201} and extended adaptive biasing force (eABF),^{202;203;204;45} In this work, the WTM-eABF algorithm was implemented within the colvars module¹⁹⁵ of NAMD 2.14.¹⁹⁴ For all of the simulations, the WTM bias temperature was set to 3000 K. For the eABF algorithm, the extended fluctuation, or standard deviation between the extended CV and the actual CV was set to $0.05 \text{ \AA}$. The chosen CV is the z component of the distance between the center of mass of the permeant and that of all POPC phosphorus atoms, where the z -axis is aligned normal to the membrane. The width of the bins for accumulating forces in the one-dimensional PMF calculation was set to $0.2 \text{ \AA}$. The value of the CV was written out every 1 ps. For efficiency, the reaction pathway was split into three windows, with the

center window overlapping its neighbors by 5 Å. The value of the CV was recorded from $z = -50$ to $z = 45$ Å for nicotine, and from $z = -45$ to $z = 45$ Å for varenicline.

The free-energy calculations were determined to have converged by the histogram of the sampling in each bin along the CV, and by differences in the gradient of the PMF between the first and second halves of the simulation. The PMF and the associated gradient with the corrected z -averaged restraint (CZAR) estimator²⁰³ were output by the colvars module from the eABF data. The PMF for neutral nicotine was obtained from 2.6 μ s of sampling in each window, singly protonated nicotine from 5.6 μ s in windows 1 and 3, and 5.9 μ s in window 2, doubly protonated nicotine from 4.9 μ s in each window, neutral varenicline from 0.6 μ s in each window, and finally, protonated varenicline from 0.8 μ s in windows 1 and 3, and 2.0 μ s in window 2.

The final PMFs were symmetrized about the membrane center, $z = 0$, by antisymmetrizing the CZAR gradients while weighting by the eABF sampling in each bin, and then numerically integrating. The unsymmetrized gradients, sampling histograms, and PMFs are shown in Appendix D. The error bars for the PMFs were computed from the eABF CZAR gradients and sampling histograms following the procedure described by Tse et al.³² and Chipot et al.⁴⁹ for plain ABF simulations.

Position-dependent diffusivity simulations

The diffusivity can be derived from trajectory data on the flattened free energy surface by the method of computing the mean-square deviation (MSD) of local displacements.^{1;99} In order to compute the position-dependent diffusivity profile, simulations biased with the time-independent forces computed by WTM-eABF were run for each molecule with the same protocol as detailed above. To avoid artifacts in the displacements, a single window was used over the entire reaction pathway. The diffusivity profile, $D(z)$, was calculated with the MSD

method by,^{99;1}

$$D(z, \tau) = \frac{1}{2\tau} \langle (\Delta z(\tau) - \langle \Delta z(\tau) \rangle)^2 \rangle \quad (6.27)$$

where τ is the lag time, and $\Delta z(\tau) = z(\tau) - z(0)$, under the assumption that the displacements occur on a flat free energy surface. The diffusivity profile was symmetrized about the membrane center, $z = 0$, by considering displacements $\Delta z(\tau)$ at z to be equal to $-\Delta z(\tau)$ at $-z$ when evaluating eq 6.27. The diffusivity simulations applied constant biasing forces accumulated from 2.6 μ s of sampling in each window for neutral nicotine, 4.1 μ s in window for singly protonated nicotine, 4.9 μ s in each window for doubly protonated nicotine, 600 ns in each window for neutral varenicline, and 800 ns each in windows 1 and 3, and 2.0 μ s in window 2 for protonated varenicline. The diffusivity profiles contain 0.6 μ s of sampling for neutral nicotine, 2.0 μ s for singly protonated nicotine, 1.1 μ s for doubly protonated nicotine, 0.4 μ s for neutral varenicline, and 0.9 μ s for protonated varenicline. The error bars for the diffusivity profiles are plus or minus half of the absolute value of the difference between $D(z)$ and $D(-z)$ from the unsymmetrized diffusivity, or $\pm \frac{1}{2} |D(z) - D(-z)|$.

6.4 Results and discussion

6.4.1 Potential of mean force and position-dependent diffusivity

The results of the PMF calculation for each molecule are shown in Figure 6.2. In all cases, the PMF levels off to zero around $z = \pm 35$ Å, indicating that these values of z would be reasonable limits for the integration of the ISD permeability (eq 6.1). It is worth pointing out that the quantity L in the present treatment of membrane permeation is not the size of the simulation box, but should be chosen such that $W_s(z)$ reaches a constant value at the boundaries, indicating the beginning of the bulk solvent region. The neutral forms of nicotine (Figure 6.2A, blue) and varenicline (Figure 6.2B, blue) experience essentially no barrier against permeation. In addition, significant regions of each neutral PMF are

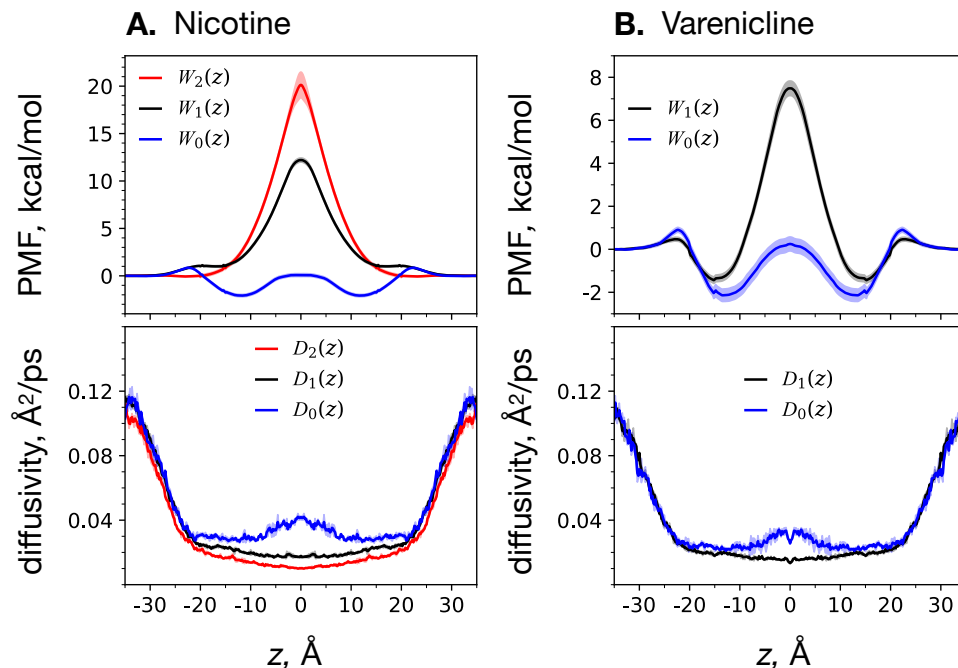


Figure 6.2: Symmetrized PMF (top) and diffusivity (bottom) of **A.** nicotine and **B.** varenicline for each protonation state (0 = neutral, 1 = singly protonated, 2 = doubly protonated). The PMF was computed by WTM-eABF enhanced sampling with the C36 additive force field. The diffusivity was computed by MSD along z with a lag time of 20 ps via eq 6.27. The PMF and diffusivity correspond to a POPC bilayer at 308 K.

below zero, corresponding to higher equilibrium probability in the POPC bilayer than in the aqueous phase. This property of neutral organic small molecules in lipid membranes has been observed before, for instance in the case of propranolol (PPL),^{186;38;205} cocaine,¹⁷³ short-chain alcohols,²⁰⁵ and ibuprofen.⁴⁰ The protonated states of nicotine (Figure 6.2A, black and red) and state of varenicline (Figure 6.2B, black) face significant barriers against permeation, as would be expected considering the more stable solvation of a charged species in the aqueous phase compared to the hydrophobic interior of a membrane, especially in the context of an additive force field.⁹⁶ The height and shape of the PMF for singly protonated nicotine are comparable to those of the protonated PPL PMF computed by Yue et al.²⁷ with umbrella sampling (US), and those of the protonated ibuprofen PMF computed by Chipot⁴⁰ with WTM-eABF.

The MSD diffusivity profiles with a lag time of 20 ps for each molecule are shown in Figure 6.2. There are differences between the singly protonated and neutral profiles for each molecule inside the membrane, but they are essentially the same in the aqueous region ($z = \pm 35$ Å). The aqueous diffusivity of doubly protonated nicotine is slightly smaller than the other two states, which can be explained by its larger hydration number, and therefore effective size, in bulk solution. Similarly, the diffusivity of each ionization state of nicotine inside the membrane tracks its local mean hydration number, such that an increase in effective size decreases its diffusivity. We also note that each diffusivity profile computed with the MSD method displayed lag time dependence, or sensitivity to the choice of τ in eq 6.27.⁴⁹ Additional results about lag time dependence, time-fractional diffusivity profiles for nicotine, and hydration numbers of each ionization state of nicotine along z are given in Appendix D. Examination of the trajectories also shows that these small molecules undergo fast isotropic diffusional dynamics on a timescale of ns for all protonation states, indicating that orientation is unlikely to be a relevant slow variable for the permeation process.

The aqueous diffusivity of about $0.115 \text{ Å}^2 \text{ ps}^{-1}$ for nicotine is slightly larger than $0.110 \text{ Å}^2 \text{ ps}^{-1}$ for varenicline, which is in line with the larger size of the latter. The experimental aqueous diffusivity of nicotine at 296 K determined with Taylor dispersion analysis is $0.0665 \text{ Å}^2 \text{ ps}^{-1}$.²⁰⁶ The factor of 1.73 increase by the simulation can be partially explained by the higher temperature of 308 K. The force field is also likely responsible, consistent with what has been observed for the self-diffusion of water, which is $0.51 \text{ Å}^2 \text{ ps}^{-1}$ for TIP3P,^{108;104} compared to the experimental $0.23 \text{ Å}^2 \text{ ps}^{-1}$.²⁰⁷ The diffusivity profiles computed by the MSD method can also be compared with other computational studies of similar molecules in POPC. For neutral PPL diffusivity computed by both Yue et al.²⁷ and Dickson et al.,³⁸ the aqueous diffusivity is around $0.05 \text{ Å}^2 \text{ ps}^{-1}$, and in the lipid-tail region the diffusivity is reduced to $0.01 \text{ Å}^2 \text{ ps}^{-1}$, which is about a factor of 2 smaller in the aqueous region and a factor of 3 smaller in the tail region, compared to neutral nicotine and varenicline.

6.4.2 Comparison of pH-partitioning and Boltzmann-weighted average potential

The specific permeability values for each ionization state of nicotine and varenicline were computed within the ISD framework via eq 6.1 using the PMFs and diffusivity profiles from Figure 6.2. According to pH-partitioning, the Henderson–Hasselbalch fraction in solution (Figure 6.1) and the specific permeability of each ionization state (Table 6.2) are sufficient to compute the effective permeability, as is presented in eq 6.2. The Henderson–Hasselbalch fraction in solution can also be represented as an offset between the PMFs of each ionization state, shown for a range of pH in the top three rows of Figure 6.3. The offsets depend on the free energy of changing the protonation state in aqueous solution, $\Delta G_{s \rightarrow s'} = \ln[10]k_{\text{B}}T(\text{p}K_{\text{a}}(s') - \text{pH})$, where the offset PMF is given by $W_s^{\text{off}}(z) = W_s(z) + \Delta G_{s \rightarrow s'}$, if $s' = s + 1$.^{27;208}

In the context of the BWAP, which is consistent with instantaneous protonation and deprotonation interconversion as in CpHMD, the PMFs of each ionization state $W_s(z)$ are combined for each pH by a Boltzmann-weighted average via eq 6.16, as shown in the bottom row of Figure 6.3. The BWAP is approximately equivalent to the result obtained by tracing the path along the lowest of the offset PMFs for each z with the lowest PMF in the aqueous bulk set to 0 in the aqueous bulk). To compute the effective pH-dependent permeability, the diffusivity profiles for each state $D_s(z)$ are also averaged, according to eq 6.17 (see Appendix D). The BWAP and averaged diffusivity are then integrated within the ISD framework via eq 6.1. Under extreme conditions of very high or very low pH, a single protonation state of the molecule is significantly populated and its PMF represents the lowest energy state for all z . In those cases, the effective permeability determined by pH-partitioning and the BWAP are essentially equivalent. For intermediate pH, the BWAP will trace the lowest energy state, generally switching states near the membrane interface.

The effective permeability of nicotine and varenicline over a range of pH computed by

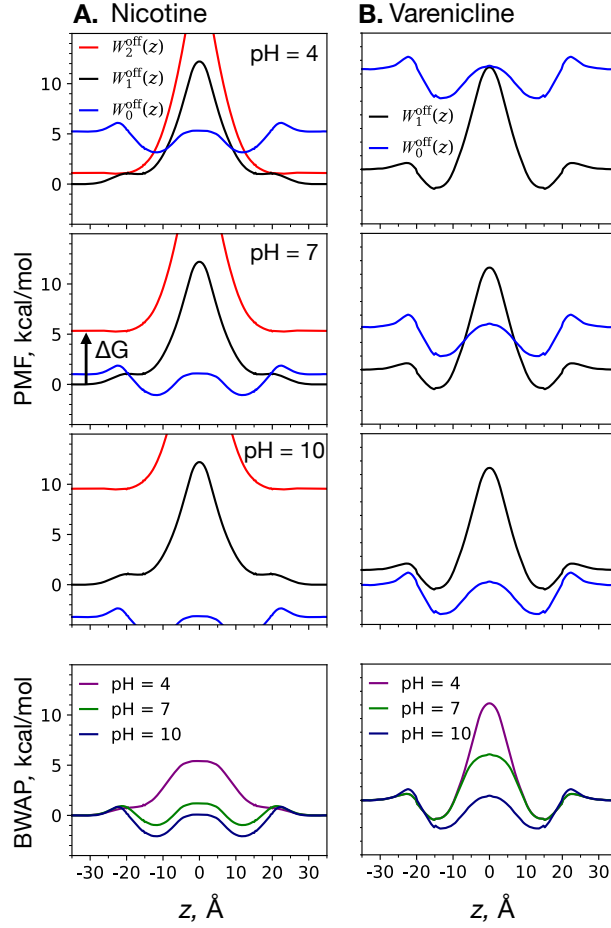


Figure 6.3: Offset PMFs (top three rows) over $\text{pH} = 4, 7$, and 10 for **A.** nicotine and **B.** varenicline in each ionization state, where in each case the singly protonated state is arbitrarily set to 0 . The offset PMFs are equal to $W_s(z) + \Delta G_{s \rightarrow s'}$, where $s' = s + 1$, and $\Delta G_{s \rightarrow s'} = \ln[10]k_B T (\text{p}K_a(s') - \text{pH})$ is the free energy of protonation in aqueous solution.^{27;208} The offsets are equivalent to that obtained by the normalized equilibrium probability density (eq 6.7). Resulting BWAP (bottom row), W_m , for the same range of pH values, computed via eq 6.16.

the two methods are depicted in Figure 6.4. In the bottom row, the ratio of the two methods shows that they are equal at very low and very high pH . This observation can be easily explained because the pH -partitioning and BWAP treatments become mathematically equivalent if a single ionization state dominates the BWAP completely over all values of z . There are significant deviations of up to more than a factor of 6 for nicotine and more than a factor of 5 for varenicline at intermediate pH . We find that the effective permeability from the BWAP is always greater than or equal to that of pH -partitioning, which is a consequence of

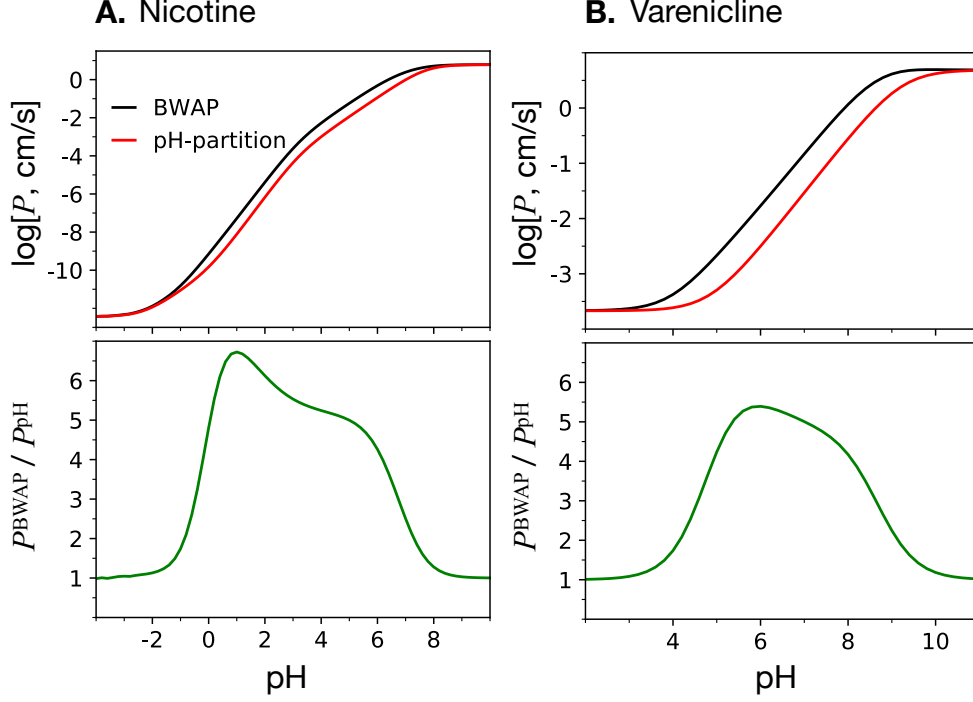


Figure 6.4: Effective permeability results based on the BWAP and pH-partitioning treatments for **A.** nicotine and **B.** varenicline. Logarithm of permeability (top) over a range of pH using the BWAP (eq 6.16) and pH-partitioning (eq 6.2) treatments. Ratio (bottom) of the permeability computed for the fast and slow limits.

the BWAP's tendency to favor the lowest energy state.

Even when more than one ionization state is present, there are circumstances in which the two treatments can yield quantitatively similar results. An example is nicotine near $\text{pH} = 7$, which occurs because its Henderson–Hasselbalch fraction is predominantly singly protonated in solution, while the PMF for the neutral, deprotonated species has the smallest free-energy barrier and dominates the effective permeability. In this case, the effective pH-partitioning permeability is given by,

$$\begin{aligned}
 P^{\text{pH-partition}} &\approx f_0 P_0 \\
 &\approx f_0 \left(\int_{-L/2}^{L/2} dz \frac{e^{+W_0(z)/k_B T}}{D_0(z)} \right)^{-1} \\
 &\approx f_0 D_0(z^\dagger) e^{-W_0(z^\dagger)/k_B T} \sqrt{\frac{W_0''(z^\dagger)}{2\pi k_B T}}
 \end{aligned} \tag{6.28}$$

On the other hand, if the BWAP (W_m) in the membrane region (close to $z^\dagger$) is dominated by the contribution from the neutral state, then the resulting effective permeability is,

$$P^{\text{BWAP}} \approx f_0 D_m(z^\dagger) e^{-W_0(z^\dagger)/k_B T} \sqrt{\frac{W_0''(z^\dagger)}{2\pi k_B T}} \quad (6.29)$$

Assuming that the diffusivity coefficients are not markedly different, we have $P^{\text{BWAP}} \approx P^{\text{pH-partition}}$. This analysis shows that the effective permeability from pH-partition and BWAP are coincidentally similar when the free energy barrier of the protonated form is much higher than that of the neutral form. More broadly, it is interesting to delineate the conditions under which the flux across the membrane is primarily determined by the passage of the neutral or protonated form of a permeant in the context of the pH-partition mechanism. Considering a situation with only two ionizable states for the sake of simplicity, it is expected that the effective permeability will be dominated by the neutral form when the pH is above a certain threshold value, because it becomes more prevalent as the pH increases ($f_0 \approx 1$). Correspondingly, when the pH is low, permeation is expected to be dominated by the more prevalent protonated form ($f_1 \approx 1$). In practice, however, the precise pH value at which the permeation dominating ionization state switches depends on a number of factors, including the pK_a of the molecule and the relative specific permeability of the neutral and protonated forms. Using a simple two state model with free energy barriers of $W_0^\dagger$ and $W_1^\dagger$ for the neutral and protonated forms, respectively, we were able to establish an empirical pH condition at which the permeation dominating ionization state switches,

$$\text{pH}_{\text{sw}} = \text{p}K_a - \frac{0.39}{k_B T} (W_1^\dagger - W_0^\dagger) \quad (6.30)$$

where 0.39 is a constant determined by a linear fit to the logarithm of permeability as a function of the free energy barrier (see Appendix D). The value of pH_{sw} defined in eq 6.30 corresponds to the pH at which the neutral and protonated contributions to the effective

permeability are equal. When the pH of the system is lower than pH_{sw} , the protonated molecule’s specific permeability and Henderson–Hasselbalch fraction will dominate the effective permeability, and neutral molecule will dominate when the pH of the system is higher than pH_{sw} . If the magnitude of the free energy barriers of the neutral and protonated forms are very close, then $\text{pH}_{\text{sw}} \approx \text{p}K_{\text{a}}$. In this case, the permeation mechanism is essentially controlled by the state with the larger Henderson–Hasselbalch fraction, f_s , present in solution. However, if the free-energy barrier of the protonated form is much larger than that of the neutral form, then pH_{sw} can be significantly smaller than the $\text{p}K_{\text{a}}$. For instance, if $W_0^\dagger \ll W_1^\dagger$, the mechanism changes when the pH is below $\text{p}K_{\text{a}} - \frac{0.39}{k_{\text{B}}T}(W_1^\dagger)$, according to the empirical analysis.

6.4.3 *Influence of ionization kinetics*

Both pH-partitioning and the BWAP treat diffusion along z as a slow variable within the ISD framework, starting from extreme views of the permeation of ionizable molecules. The protonation and deprotonation rates are assumed to be either very slow (pH-partitioning) or extremely fast (BWAP). Ultimately, neither approach accounts for how ionization kinetics are coupled to the permeation process.

A simple argument suggests that changes in ionization state may indeed occur too slowly to interfere with the spatial diffusion of the molecule in solution. Based on the diffusivity of nicotine and varenicline in solution (Figure 6.2), the average root-mean-square displacement ($\sqrt{2Dt}$) on the timescale of protonation at $\text{pH} = 7$ (~ 1 ms) is on the order of 1 μm . This is a remarkably large length scale compared to the thickness of the membrane. On this lengthscale, the bulk solution essentially behaves as a mixture with fixed concentrations of the different protonation states. However, this argument does not account for the interplay of ionization kinetics and membrane translocation.

To assess the physical validity of approximate treatments such as pH-partitioning and

the BWP in the context of membrane crossing events, it is useful to examine the permeation process of ionizable molecules within the powerful framework provided by TPT.^{67;17;68;69;70;18} The pH-partition treatment is obtained by setting the transition rates $k_{s',s}(z)$ to zero in the SME (eq 6.6), keeping only the spatial diffusion along z as part of the dynamics. In this case, the committor, $q_s^{\text{diff}}(z)$, is exactly given by eq 6.25.^{76;18} It may be worth emphasizing that, while committor analysis is often associated with the ‘true’ kinetics determined from unbiased MD trajectories,^{209;210;211} the present analysis follows from the diffusive stochastic Brownian dynamics implied by the Smoluchowski master equation (eq 6.6). Although this is an approximation of the true dynamics, it can be used to shed light on the overall timescale relevant to membrane permeation of an ionizable molecule. For instance, it is possible to express in closed-form the mean reactive transit time during a diffusive transition of the permeant across the membrane while remaining in ionization state s as,^{76;18;67}

$$\langle \tau_{\text{r}} \rangle = \frac{1}{J_s} \int dz q_s^{\text{diff}}(z) (1 - q_s^{\text{diff}}(z)) \pi_s(z) \quad (6.31)$$

It is interesting to examine how $\langle \tau_{\text{r}} \rangle$ compares with the timescales associated with the discrete transition rates k_{on} and k_{off} . The results given in Table 6.1 indicate that even the longest mean transit times are much shorter than the expected lifetime of a given ionization state. When $\text{pH} = 7$, k_{on} is about 10^3 s^{-1} , which translates to a timescale of 1 ms, considerably longer than the mean reactive transit times. For singly protonated nicotine ($\text{p}K_{\text{a}} = 7.72$), k_{off} is $10^{2.28} \text{ s}^{-1}$, which translates to a timescale of about 5 ms. The transit time for singly protonated nicotine is only 7.15 ns, suggesting that it has enough time to fully translocate without deprotonation. For varenicline ($\text{p}K_{\text{a}} = 9.22$), k_{off} translates to a deprotonation timescale of 0.15 s, which is even slower. Based on these considerations, it appears unlikely that these or similar ionizable molecules would alter their ionization states during the course of such relatively short permeation events.

On the basis of TPT, the net reactive flux from the Smoluchowski master equation can

Table 6.1: Mean reactive transit times, $\langle\tau_r\rangle$, ns

Molecule	$\langle\tau_r\rangle$
neutral nicotine	167
singly protonated nicotine	7.15
doubly protonated nicotine	8.14
neutral varenicline	236
singly protonated varenicline	73.4

be expressed in terms of the committor probability.^{67;17;68;69;70;18} This provides a useful starting point to formulate a perturbative approach that exploits the pH-partitioning treatment as a reference model to quantitatively characterize the effect of ionization kinetics on permeation. The contribution to the net reactive flux arising from the z -dependent protonation/deprotonation transition rates, $k_{s',s}$, expressed in terms of the committor of the unperturbed reference model is,

$$J^{\text{trans}} = \sum_{s',s} \int_{-L/2}^{L/2} dz \Theta(q_{s'}^{\text{diff}}(z) - q_s^{\text{diff}}(z)) (q_{s'}^{\text{diff}}(z) - q_s^{\text{diff}}(z))^2 k_{s',s}(z) \pi_s(z) \quad (6.32)$$

where the factor of $1/2$ was removed by exploiting the symmetry of the integrand (imposed by the Θ function). Assuming that the permeating molecule has only the deprotonated ($s = 0$) and singly protonated ($s = 1$) states, with the rates $k_{1,0} \equiv k_{\text{on}}$ and $k_{0,1} \equiv k_{\text{off}}$, the perturbative contribution to the net reactive flux is expressed as $J^{\text{trans}} = \delta J_{\text{off}} + \delta J_{\text{on}}$, where,

$$\delta J_{\text{off}} = \int_{-L/2}^{L/2} dz \Theta(q_0^{\text{diff}}(z) - q_1^{\text{diff}}(z)) (q_0(z) - q_1(z))^2 k_{\text{off}} \pi_1(z) \quad (6.33)$$

and

$$\delta J_{\text{on}} = \int_{-L/2}^{L/2} dz \Theta(q_1^{\text{diff}}(z) - q_0^{\text{diff}}(z)) (q_1(z) - q_0(z))^2 k_{\text{on}} \pi_0(z) \quad (6.34)$$

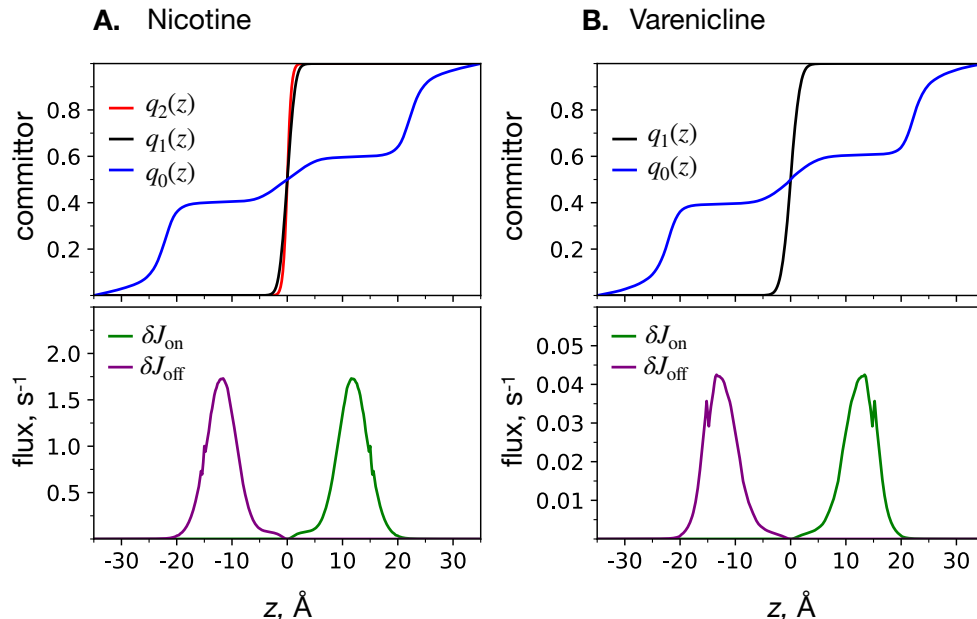


Figure 6.5: Committors (top) of each protonation state across the membrane based on eq 6.25, and the components δJ_{off} and δJ_{on} (bottom) of the deprotonation/protonation contribution to the overall steady-state flux for **A.** nicotine and **B.** varenicline. The value of the committor represents the probability that the molecule will cross to the right before returning to the left, given its current position. The component δJ_{on} is calculated with eq 6.34, with $k_{\text{on}} = 10^3 \text{ s}^{-1}$, corresponding to the protonation rate in bulk solution at pH = 7, and the component δJ_{off} is calculated with eq 6.33. Note that the doubly protonated state of nicotine was not included in the committor flux model for the sake of simplicity.

The transition rate k_{on} is a constant and k_{off} depends on z according to the $\text{p}K_{\text{a}}$ via eq 6.5.

The first term δJ_{off} yields a positive contribution arising from deprotonation (k_{off}) when $q_0 > q_1$, and the second term δJ_{on} yields a positive contribution arising from reprotonation (k_{on}) when $q_1 > q_0$. These two processes occur in the correct order for a molecule permeating from the left to the right: the deprotonation (k_{off}) occurs on the left of the membrane (positive flux contribution), and the reprotonation (k_{on}) occurs on the right of the membrane (positive flux contribution). The result of this analysis is illustrated in Figure 6.5. The contributions of each to the total transition flux show that the deprotonation/protonation transitions occur near the two interfaces. However, Figure 6.6 shows that these transitions give rise to a very small total contribution, $\delta J_{\text{off}} + \delta J_{\text{on}}$, to the net flux, relative to the pH-partitioning treatment. It is also worth emphasizing that this result was

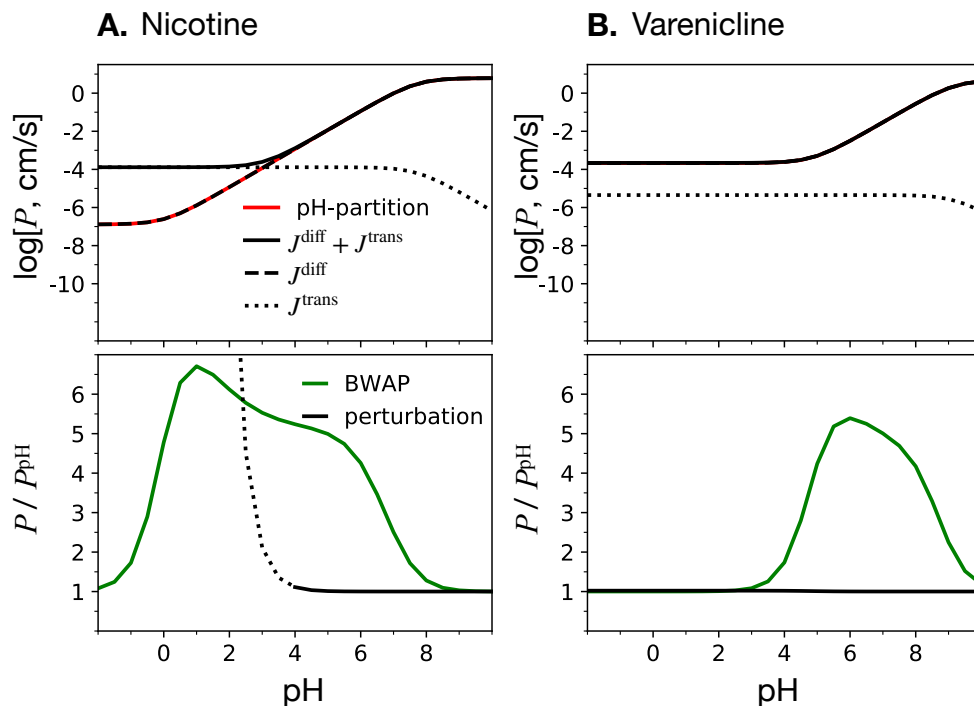


Figure 6.6: Logarithm of permeability versus pH for each component of the total reactive flux (top) and ratio of the permeability derived from the BWAP and perturbed reactive flux to the pH-partitioning treatment for **A.** nicotine and **B.** varenicline. Top: The perturbed reactive flux permeability (solid, eq 6.22) is shown in its individual components, where the diffusive component (dashed, eq 6.23) tracks the pH-partitioning permeability, and the state transition component (dotted, eq 6.24) reaches a constant value at low pH. Bottom: The permeability ratio relative to the pH-partitioning treatment is shown for the perturbed reactive flux approach (black line) and BWAP (green line). A ratio close to 1 indicates agreement with pH-partitioning. For nicotine, the ratio is displayed as a dotted line when the perturbative approach becomes invalid because the protonation rate is too large (below $\text{pH} = 4$).

obtained using a uniform k_{on} , corresponding to the protonation rate in bulk solution at $\text{pH} = 7$ (Figure 6.5). The contribution of protonation/deprotonation kinetics to the next flux would be even smaller using a reduced transition rate in the membrane region where the water density is lower. Essentially, the physical picture underlying the BWAP is incorrect because ionization kinetics are too slow on the timescale of membrane crossing to affect the permeation of small ionizable molecules such as nicotine and varenicline. For varenicline, the perturbative treatment indicates that the pH-partitioning method dominates at all pH. For nicotine, the perturbative treatment becomes invalid below a pH of 4 because the protonation rate becomes too large (dotted line in bottom plots of Figure 6.6).

To better visualize the significance of the perturbation of ionization kinetics to the net reactive flux, we simulated the permeation of the neutral and singly protonated states of nicotine as a combined Brownian dynamics and Markov state model (BD-MSM), which allows transitions between discrete states.⁹ The stochastic BD-MSM trajectory is generated from the Markovian propagator $\mathcal{P}_\tau$ defined by eq 6.20 for the continuous diffusive and discrete hopping steps, $\{z(t), s(t)\} \rightarrow \{z(t + \tau), s(t + \tau)\}$. The results of the permeation BD-MSM simulations of nicotine at pH = 7 and $T = 308$ K are shown in Figure 6.7. The average of the estimated permeability computed for 100 replicate trajectories is shown in Figure 6.7B. The permeability for each individual trajectory was computed by,¹

$$P(t) = \frac{N_c(t)}{2t \sum_s \pi_s(-L/2)} \quad (6.35)$$

where $N_c(t)$ is the number of crossings (in either direction) up to time t , and $\pi_s(-L/2)$ is the normalized equilibrium aqueous concentration of ionization state s (see eq 6.7). The aqueous fraction of each ionization state in eq 6.7 was computed individually for each trajectory. To approximate an equilibrium ensemble, 16 simulations were initiated in the deprotonated state, and 84 were initiated in the singly protonated state, according to the Henderson–Hasselbalch fractions for pH = 7.

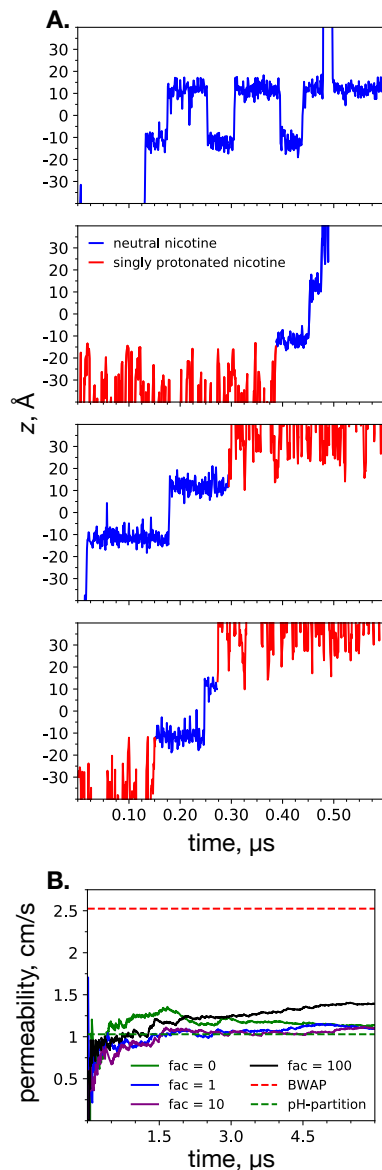


Figure 6.7: **A.** Typical permeation trajectories of nicotine at pH = 7 generated by combining Brownian dynamics with Markov state model (BD-MSM) for allowing transitions between different discrete states.⁹ The segments of the trajectory corresponding to a neutral deprotonated and charged protonated nicotine are shown in blue and red, respectively. The protonation/deprotonation rates were multiplied by a factor of 100 to increase the probability of capturing the events depicted in the figure. From top to bottom: a neutral crossing, deprotonation and subsequent neutral crossing, protonation following neutral crossing, and sequential deprotonation–neutral crossing–protonation. **B.** Permeability computed by counting crossings¹ in the combined BD and Markov model for several ionization kinetics accelerating factors (fac) at pH = 7 (fac = 0 does not allow switching states, fac = 1 uses the experimental rate).¹⁷² Each permeability is the mean of 100 trajectories, for which 16 were initiated in the neutral state and 84 were initiated in the protonated state. The BD was run with a timestep of 0.01 ps in 1-dimension with periodic boundaries at ± 40 Å. The position dependent forces, diffusivity and its derivative, and local k_{off} were computed by linear interpolation from the computed PMFs and diffusivity profiles.

Within the BD-MSM model, switches are included between the deprotonated and protonated ionization states, translating to BD integration with a state dependent external potential and diffusivity. At each time step, the state is switched if $k_{s',s}(z)\Delta t$ is greater than or equal to a uniform random number.⁹ Again, the rate k_{on} is a constant that depends only on the pH, while k_{off} has z dependence controlled by the $\text{p}K_{\text{a}}$ (eq 6.5). In order to assess the effect of the rate of ionization kinetics on the observed permeability, separate BD-MSM ensembles were simulated with the experimental interconversion rates¹⁷² multiplied by a factor (fac) of 0, 1, 10, and 100. When the interconversion rates are zero (the multiplying factor is 0), the system formally obeys the pH-partition hypothesis. We observed that when the multiplicative factor is 1 or 10, the estimated permeability is essentially equivalent to the pH-partition effective permeability. When the multiplicative factor is 100, the permeability begins to deviate from pH-partitioning. Additional BD-MSM simulations indicate that the BWAP limiting behavior is approached only when the multiplying factor is 10^6 or larger (not shown). For the sake of clarity, the trajectories shown in Figure 6.7A used interconversion rates multiplied by a factor of 100 to increase the probability of capturing the specific modes of crossing. Permeating trajectories of the neutral molecule are the most frequently observed, despite its smaller Henderson–Hasselbalch fraction f_0 . On the other hand, the protonated-neutral-protonated trajectories, which are implied by the BWAP, are exceedingly rare.

6.4.4 *Comparison with experiments and previous calculations*

The specific permeability values for each permeant computed within the ISD framework via eq 6.1 using the PMF and diffusivity profile from Figure 6.2 are shown in Table 6.2, with a comparison to experiment in the context of pH-partitioning. The computational specific permeability values (which are strictly pH independent) in units of cm/s are 6.12, 1.28×10^{-7} , and 3.70×10^{-13} for neutral, singly protonated, and doubly protonated nicotine, respectively,

Table 6.2: Specific POPC membrane permeability to each ionization state of nicotine and varenicline at 308 K, cm/s

Molecule	ISD	Expt. ^a
neutral nicotine	6.12	1.331×10^{-5}
singly protonated nicotine	1.29×10^{-7}	3.603×10^{-7}
doubly protonated nicotine	3.70×10^{-13}	7.231×10^{-8}
neutral varenicline	4.88	—
singly protonated varenicline	2.14×10^{-4}	—

a. Experimental apparent specific permeability for nicotine from ref. 20, determined from pig cheek cells.¹⁶⁸

and 4.88 and 2.14×10^{-4} for neutral and singly protonated varenicline. In the interest of comparing the specific permeability of nicotine and varenicline with previous computational work that uses the ISD framework, we consider propranolol (PPL), a weak base with similar lipophilic properties.^{27;38;186} For neutral PPL permeability, Dickson et al. report 17.4 cm/s, determined by US with a POPC membrane at 303 K, and modeled with the AMBER Lipid 14 force field.^{38;212} Swift and Amaro report 1×10^{-6} cm/s, determined by US with a DMPC membrane at 310 K, and modeled with CHARMM36.¹⁸⁶ The PMFs of Swift and Amaro,¹⁸⁶ and Dickson et al.^{38;212} are qualitatively similar. Therefore, we suspect that the considerable discrepancy is due to an error in unit conversion. Yue et al.²⁷ report 3.16×10^{-3} cm/s for the neutral form and 4.17×10^{-8} cm/s for the protonated form, determined by US with a POPC membrane at 310 K, and modeled with CHARMM36. The discrepancy for the neutral form of PPL in the work of Yue et al. can be attributed to a qualitatively different PMF from that of Swift and Amaro, Dickson et al., and the present work. The protonated form of PPL from Yue et al.²⁷ is characterized by a PMF and permeability comparable to those of singly protonated nicotine.

Reconciling computational permeability values with experimental measurements in the case of ionizable molecules remains challenging.^{213;21;214;206;215;23;170;40} Measurement of the apparent specific permeability of lipophilic small molecules, such as the neutral forms of many ionizable drugs, is confounded by additional rate-controlling barriers that are distinct

from the permeability of the membrane.^{215;170;216;217} In the common experimental setup of imposing a steady-state flux across a layer of cells (such as Caco-2),^{21;214} the apparent permeability, P_{app} , will be upper-bounded by the aqueous boundary layer²¹⁶ (ABL) and lower-bounded by paracellular pathways, each with their own respective permeability, P_{ABL} and P_{para} .²¹⁵ The P_{ABL} term combines in series with the membrane permeability, while P_{para} combines in parallel, such that the apparent permeability is given by,²¹⁵

$$\frac{1}{P_{\text{app}}} = \frac{1}{P + P_{\text{para}}} + \frac{1}{P_{\text{ABL}}}. \quad (6.36)$$

Around neutral pH, the specific permeability of the neutral form of the molecule tends to dominate, such that, $P_0 f_0 \gg P_{s \neq 0} f_{s \neq 0}$, and eq 6.2 becomes $P \approx P_0 f_0$. In this case, $P_0 f_0$ is generally also much larger than P_{para} and P_{ABL} . The apparent permeability of multiple steroids that are lipophilic and neutral is dominated by P_{ABL} , with an average value around $10^{-4.7}$ cm/s,²¹⁵ which is orders of magnitude smaller than predictions by MD simulations,³² demonstrating the gravity of the ABL. This trend extends to practically all small neutral molecules with membrane permeability measured across cell layers. Under extreme pH conditions in which f_0 is negligibly small, P_{app} will depend on the specific permeability of one or more charged species, which could be comparable to or much smaller than P_{para} and P_{ABL} . In such cases, P_{obs} is confounded by the lower bound of P_{para} .^{215;168}

The Caco-2 permeability of nicotine at 310 K and pH = 7.4 is $1.94 \pm 0.49 \times 10^{-5}$ cm/s.²¹⁴ The corresponding computational pH-partitioning effective permeability is 1.98 cm/s, while the BWAP effective permeability is 3.66 cm/s. For varenicline, the permeability at 310 K with pH = 7.4 is 5.04×10^{-5} cm/s through vinblastine-exposed Caco-2 (VB-Caco-2) and 4.73×10^{-5} cm/s through MDCK-MDR1.¹⁵⁸ The pH-partitioning result is 0.073 cm/s, and the BWAP result is 0.35 cm/s. The Caco-2 and MDCK studies are affected by the ABL, which is not accounted for in the ISD framework.^{218;215} Permeability through the ABL can be approximated by $P_{\text{ABL}} = D_{\text{aq}}/h_{\text{ABL}}$, consistent with the homogeneous solubility-diffusion

model,²¹⁹ where D_{aq} is the aqueous diffusivity and h_{ABL} is the height of the ABL.²¹⁸ Using the nicotine experiment as an example, a 1.5 mL cylindrical cell holder with a 1.2 cm radius will present an h_{ABL} of around 0.33 cm.²¹⁴ Neglecting the 100 rpm stirring velocity, and using the experimental aqueous diffusivity of nicotine,²⁰⁶ P_{ABL} for this study is around 2×10^{-5} cm/s, accounting for nearly the entire apparent permeability.

Experimentalists generally assume pH-partitioning (eq 6.2),^{161;168} which allows for the determination of apparent specific permeability of ionizable molecules. As an illustration, Chen et al.¹⁶⁸ determined the apparent specific permeability of each ionization state of nicotine by measuring the apparent permeability through pig cheek cells over a range of pH, and then solving eq 6.2 for each P_s . These results are summarized in Table 6.2. The poor agreement for neutral nicotine can be attributed to the rate-limiting ABL.²¹⁵ The poor agreement for doubly protonated nicotine is likely due to paracellular pathways through the cheek cells, effectively placing a lower bound on the apparent permeability.^{215;168} The experimental permeability of the singly protonated state agrees well with the ISD result, indicating that permeability around this order of magnitude sits between the upper ABL and the lower paracellular limiting cases, allowing for more direct comparison with simulation. This agreement also supports the validity of the pH-partitioning treatment under these experimental conditions.

More experimental support for pH-partitioning is provided by LUV fluorescence experiments, which usually have negligible ABL effects, no paracellular contributions, and have the added advantage of presenting single-component bilayers.^{27;170;23;25} For example, Eyer et al.²³ determined the apparent specific permeability of neutral propranolol to be 1.55 cm/s using pH-sensitive LUV fluorescence with POPC liposomes of 88 nm radius at 298 K. It has been shown by Sezer and Oruç with kinetic models, however, that ionization kinetics become rate-limiting when measuring the permeability of ionizable molecules with high neutral specific permeability.²⁵ This phenomenon is related to the surface area to volume ratio of the

LUV, and the method of detecting permeation. For LUVs with a high surface area to volume ratio, permeation of the neutral molecule occurs faster than the fluorescent dye can change its ionization state, resulting in a signal that is insensitive to the neutral specific permeability.^{25;170} Sezer concludes that under the conditions of the experiment, a neutral specific permeability greater than 0.5 cm/s cannot be resolved.²⁵ The apparent specific permeability of neutral propranolol reported from the LUV experiment is therefore no more than a lower limit on the true value. It follows that similar studies with larger liposomes might yield more precise neutral specific permeability estimates, but in reality the effect of the ABL is expected to reappear.¹⁷⁰ The apparent permeability of formic acid was found to be ten times faster when determined with LUVs²²⁰ than with giant unilamellar vesicles (GUVs),²²¹ although there are confounding variables between the studies, such as buffering.¹⁷⁰

Another experimental method that largely negates the effects of the ABL, this time with planar bilayers, is the so-called reaction layer,^{170;222;223} which was used by Gutknecht et al.²¹³ to determine the apparent permeability of carbon dioxide to be 0.35 cm/s. When the ABL is saturated by HCO_3^- in the presence of carbonic anhydrase (CA), the rate-limiting step becomes the permeation of carbon dioxide due to the speed of the catalyzed reaction.^{213;170} Using scanning electrochemical microscopy with the reaction layer method, Missner, Pohl, et al.²²⁴ determined a lower-limit permeability of 3.2 cm/s for carbon dioxide at 310 K. Hub et al.²²⁵ found from MD simulations of carbon dioxide in a 1:1 POPE:POPC membrane a permeability of 3.0 cm/s at 300 K. It follows that the ability of the reaction layer to increase the lower observable limit of permeability beyond that of LUV fluorescence is a consequence of the fast reaction catalyzed by CA compared to the slow change in ionization state of the fluorescent molecule, underlining the importance of separating the timescales of permeation and ionization kinetics.

6.5 Conclusions

We computed the PMF and diffusivity for nicotine and varenicline in each of their ionization states across a POPC membrane with biased MD. We calculated effective permeability coefficients according to either the pH-partition or BWAP treatments, each within the ISD framework. Both are approximate treatments: pH-partition assumes that the rate of changing ionization state is negligible on the timescale of permeation, while BWAP assumes that changes in ionization state occur instantaneously.

In an effort to assess the influence of ionization kinetics on the rate of permeation, we developed a perturbative approach in the context of TPT based on the Smoluchowski master equation to estimate the contribution to the net reactive flux from changes in ionization state. Changes in ionization state are treated as small perturbations that occur at the experimental rates of deprotonation and protonation. The results of this analysis leads us to the conclusion that pH-partitioning is the more physical way to treat the permeation of small ionizable molecules. BWAP does not provide a correct physical picture for the small ionizable molecules considered in this work because the rates of changing ionization states are slow compared to the computed rates of permeation. On the other hand, this approximate treatment could be appropriate for modeling the slow permeation of large ionizable molecules (e.g., peptides and toxins) which encounter considerable free energy barriers for all of their ionization states. In the case of the peptide WWWK flanked with two positive charges (protonated lysine and amidated C-terminus), Elber estimated a permeation time of 5 s using milestoning.⁶⁰ Given the slow timescale of the permeation process, averaging over changes in ionization state along the z coordinate across the membrane would be a reasonable assumption.

Interpreting experimental measurements in the case of ionizable molecules is difficult, as the apparent permeability can be affected by a number of hidden factors that obscure the permeability of the membrane itself to the different ionization states of the molecule. Nev-

ertheless, the results of the computations are moderately encouraging based on the limited experimental data. For the singly protonated state of nicotine, the computational results agree well with the experimental measurements.¹⁶⁸ However, the agreement for neutral and doubly protonated nicotine is poorer. The discrepancy is likely due to factors associated with the aqueous boundary layer (neutral form) or leaks through paracellular pathways (doubly protonated form). There are no measurements in the case of varenicline, but the high molecular similarity with nicotine suggests that the computational results should be reasonable. While we are confident that our enhanced sampling calculations are well-sampled and converged (see Appendix D), we note that even a small error in the PMF due to the use of the additive CHARMM36 force field or choice of z as a reaction coordinate could result in a significant difference between the already imprecise experimental measurement and the computational permeability. Notably, the use of a polarizable force field has been shown to be especially important for simulating ionic species in membranes, resulting in lower barriers to permeation.⁹⁶

Looking ahead, we are interested in understanding the importance of induced polarization phenomena when determining membrane permeability to small molecules. Recent results of POPC membrane permeability to water computed using the Drude2023 polarizable force field for lipids^{30;81} and the SWM4-NDP water model⁸⁰ show improved agreement with experiment compared to previous results obtained with the additive CHARMM36 force field.³⁰ While the free energy barrier against water crossing the POPC lipid bilayer is smaller with the polarizable model,¹ the self-diffusion of water is slower,^{108;104} indicating that we might expect some degree of cancellation of errors in the determination of membrane permeability to small molecules with the additive model, which has been observed by Comer et al.¹⁸⁷ Employing the more rigorous polarizable model for the computation of membrane permeability to small ionizable molecules from first principles does seem to be a compelling future direction.

CHAPTER 7

PERMEABILITY OF PROTONATED NICOTINE

ABSTRACT – The permeability of the protonated form of nicotine is computed with the reactive flux formulation and short unbiased trajectories. Protonated nicotine is an example of a permeation process of a charged molecule for which membrane deformation is non-negligible. The validity of the ISD model is assessed by comparison with the rate obtained by the reactive flux. The permeation pathway is optimized via the mean force string method. This approach illustrates the challenge of evaluating the transition rate of a system with an approximate reaction coordinate.

7.1 Introduction

The theory of biological membrane permeability has been developed primarily for the application of drug design.^{19;20} In common practice, a relevant measure of the permeability of drugs taken orally is assumed to be that of Caco-2 cells,²¹ which model human intestinal lining. Membrane permeability has also been modeled by molecular dynamics (MD) simulations,⁶ which generally involves computing the potential of mean force (PMF)¹⁰ and the position-dependent diffusivity⁸⁴ for estimating the permeability in terms of the closed-form expression of the inhomogeneous solubility-diffusion (ISD) model.⁸ Efforts to fully automate the calculation of the ISD permeability from enhanced sampling MD simulations and the training of neural networks to predict and optimize small molecule permeability have been undertaken.²²⁶ Many drugs, however, may not be treated in such a straightforward way. A significant percentage of drugs are ionizable, such as all alkaloids. In these instances, estimating the permeability is more complex, due to a combination of protonation kinetics^{27;25;26;23} and memory effects.^{96;59}

To examine the the physical picture of the permeation of small ionizable molecules more

deeply, we return to the example of nicotine.²⁶ Nicotine, as an alkaloid, is weakly basic, with two titratable nitrogen atoms. The pK_a of each ionization state is 7.72 for the singly protonated state and 3.22 for the doubly protonated state.¹⁵⁶ According to the pH-partition hypothesis, each ionization state of nicotine permeates the membrane in parallel, each with its own specific permeability coefficient.¹⁶⁸ The effective permeability of the ensemble is then the sum of each specific permeability, weighted by the pH-dependent Henderson-Hasselbach fraction of each ionization state in the bulk solution. The pH-partition hypothesis has been supported by kinetic modeling^{23;25} of liposome fluorescence experiments and transition path theory (TPT)²⁶ using experimental rates of protonation.¹⁷² Interestingly, different sources deliver nicotine to the body under different conditions: pH 5.5 from burning tobacco, physiological pH from nicotine gum, to pH 8 from freebase vapes.²²⁷ The high population of singly protonated nicotine at acidic and neutral pH warrants an accurate estimate of the specific permeability, despite its comparatively small magnitude.

It is currently understood that the key assumptions of the ISD model generally do not hold for charged molecules, due to slow orthogonal degrees of freedom, related to membrane deformation.^{96;59} Approaches to handle the permeation of ionized species (molecules, ions, proteins) include the addition of an orthogonal degree of freedom in a 2-dimensional PMF,²⁷ an ion-induced defect mechanism,⁹⁶ and Milestoning.⁶⁰ Modeling the permeation process in the presence of membrane defects could potentially be achieved by the parameterizing a committor consistent string pathway.¹⁵² The method of artificial intelligence-guided transition path sampling²²⁸ which was applied to lipid flip flop, also seems like it could be employed for ionizable molecule permeation.

To treat the transition pathway of nicotine across a membrane, we begin with the application of the mean force string methods in the space of four relevant collective variables (CVs). In the interest of obtaining the transition rate in a transparent and straightforward way, we reduced the dimensionality in CV space to two.

We begin with a long steered MD simulation for the recording of candidate CVs. We then proceed to use the mean force string method to optimize the pathway in the space of four significant CVs. In order to extract the exact rate of barrier crossing with short unbiased trajectories, we further narrowed down the space of CVs to 2: The signed difference in coordination number of lipid headgroups from the lower and upper leaflets (ξ), and z . With 2 CVs, it is possible to apply the analysis of Hinsen and Roux²²⁹ to extract the transmission coefficient with short unbiased trajectories. For this analysis, z was chosen as the primary CV due to its fundamental significance for defining the two sides of the membrane over the entire reaction coordinate. The remaining ξ is the auxiliary CV in the transmission coefficient analysis. Importantly, the coordination to the upper and lower leaflets is expected to have the largest impact on the permeation process near the center of the membrane.

7.2 Theoretical developments

7.2.1 Reactive flux formulation of permeability

The permeability based on the derivation of the reactive flux time-correlation function of Chandler is given by,^{66;13}

$$\begin{aligned}
P &= \lim_{t \rightarrow \tau} \frac{d}{dt} \langle H_a(0) H_b(t) \rangle \frac{1}{\pi(-L/2)} \\
&= \langle H_a(0) \dot{H}_b(\tau) \rangle \frac{1}{\pi(-L/2)} \\
&= -\langle \dot{H}_a(0) H_b(\tau) \rangle \frac{1}{\pi(-L/2)} \\
&= \frac{\langle \delta(z - z^\dagger) \dot{z}, H_b(\tau) \rangle \langle \delta(z - z^\dagger) \rangle}{\langle \delta(z - z^\dagger) \rangle \pi(-L/2)} \\
&= \langle \dot{z} H_b(\tau) \rangle_{z^\dagger} \frac{\pi(z^\dagger)}{\pi(-L/2)}
\end{aligned} \tag{7.1}$$

where H_a and H_b are indicator functions equal to 1 or 0 that can serve to monitor the population of the reactant or product of the permeation process, τ is the Markovian lag time, $\dot{z}$ is the instantaneous velocity at $t = 0$, $\pi(z)$ is the equilibrium probability distribution, L is the thickness of the membrane, and $z^\dagger = 0$ is the center of the membrane.

An attractive modification of the permeability coefficient expressed from a reactive flux time-correlation function is to incorporate the committor probability, resulting in less noise and convergence at shorter lag times. To begin, we define the indicator functions H_a and H_b on the basis of the committor probability q . The value of the indicator functions is 1 or 0, depending on whether q is larger or smaller than $q^\dagger$, respectively. It makes sense to choose $q^\dagger = 0.5$, although this is not a formal necessity. correspondingly, we have that $q(z^\dagger) = q^\dagger$. The permeability expressed in terms of a committor-weighted reactive flux time-correlation function is given by,^{18;13}

$$\begin{aligned}
P &= \lim_{t \rightarrow \tau_q} \frac{d}{dt} \langle H_a(0) H_b(t) (q(t) - q(0)) \rangle \frac{1}{\pi(-L/2)} \\
&= \langle H_a(0) \dot{H}_b(\tau_q) (q(\tau_q) - q(0)) \rangle \frac{1}{\pi(-L/2)} \\
&= -\langle \dot{H}_a(0) H_b(\tau_q) (q(\tau_q) - q(0)) \rangle \frac{1}{\pi(-L/2)} \\
&= -\frac{\langle \delta(q - q^\dagger) \dot{q}(0) H_b(\tau_q) (q(\tau_q) - q(0)) \rangle}{\langle \delta(q - q^\dagger) \rangle} \frac{\langle \delta(q - q^\dagger) \rangle}{\pi(-L/2)} \\
&= \langle \dot{q}(0) H_b(\tau_q) (q(\tau_q) - q(0)) \rangle_{q^\dagger} \frac{\pi_q(q^\dagger)}{\pi(-L/2)}
\end{aligned} \tag{7.2}$$

where

$$\langle \delta(q - q^\dagger) \rangle = \pi_q(q^\dagger) = \pi(z^\dagger) \left(\frac{dz}{dq} \right)_{q^\dagger} \tag{7.3}$$

is the equilibrium probability distribution of the committor function.

7.2.2 *Enhanced sampling of auxiliary collective variable at the top of the barrier*

The transmission coefficients described in the previous section may not converge well if significant deviations of the true pathway from the chosen reaction coordinate occur near the top of the barrier. For example, if there is a significant membrane deformation that occurs as the molecule crosses $z = z^\ddagger$ and $q = q^\ddagger$, prohibitively extensive sampling would be needed for the indicator and committor functions to give an accurate representation of the transition from a to b. This complication has been addressed by Hinsen and Roux²²⁹ by introducing an auxiliary CV and computing its PMF when the primary CV z is fixed at $z^\ddagger$. In the case of the membrane permeation of a molecule with a net charge, we determined that an ideal choice of the orthogonal degree of freedom is the difference in coordination number of upper and lower leaflet phosphorus atoms, ξ . The PMF of ξ , where z is fixed to $z^\ddagger$ by strong harmonic restraints, was computed via umbrella sampling. Given the PMF of ξ , the permeability coefficient expressed as a reactive flux time-correlation function is given by,

$$P = \frac{\pi(z^\ddagger)}{\pi(-L/2)} \int d\xi \pi(\xi; z^\ddagger) \langle \dot{z} H_b(\tau) \rangle_{z^\ddagger, \xi} \quad (7.4)$$

where $\langle \dot{z} H_b(\tau) \rangle_{z^\ddagger, \xi}$ is the conditional average of trajectories started at the transition state $z^\ddagger$ with an initial value of the orthogonal degree of freedom equal to ξ and,

$$\pi(\xi; z^\ddagger) = \frac{e^{-W(\xi, z^\ddagger)/k_B T}}{\int d\xi e^{-W(\xi, z^\ddagger)/k_B T}} \quad (7.5)$$

is a slice of the 2-dimensional probability of z and ξ where z is restrained to $z^\ddagger$. Similarly, the permeability coefficient expressed as a committor-weighted reactive flux time-correlation

function is given by,

$$P = \left(\frac{dz}{dq} \right)_{q^\dagger} \frac{\pi(z^\dagger)}{\pi(-L/2)} \int d\xi \pi(\xi; z^\dagger) \langle \dot{q} H_b(\tau_q) (q(\tau_q) - q(0)) \rangle_{z^\dagger, \xi} \quad (7.6)$$

where $\langle \dot{q} H_b(\tau_q) (q(\tau_q) - q(0)) \rangle_{z^\dagger, \xi}$ is the conditional average of trajectories that begin at $z^\dagger$ and ξ . In this case, the conditional average at $q^\dagger$ is the same as a conditional average at $z^\dagger$ because $q(z^\dagger) = q^\dagger$.

7.3 Simulations and Methods

7.3.1 *The mean-force string method of collective variables*

The mean force string method was applied to four collective variables: 1) the standard z -component of the center of mass distance between the permeant and the membrane, 2) the coordination number between the headgroup phosphorus atoms of lower leaflet and the permeant, 3) the coordination number between the headgroup phosphorus atoms of the upper leaflet and the permeant, and 4) the z -component between the permeant's center of mass and center of charge (acidic proton). The coordination numbers were calculated using the colvar module of NAMD with default settings. The cutoff distance was set to 8 Å, based on the square root of the area per lipid, or the approximate average distance between lipids on the xy -plane of the membrane. The coordination numbers were calculated with respect to the acidic proton of the permeant.

Both the choice of the four collective variables and the initial configurations for each of the mean force string method starting windows were obtained by a steered-MD simulation of nicotine dragged along z across the POPC membrane. The steered-MD proceeded from $z = -40$ Å to $+40$ Å over 1 μ s. The force constant on z was set to 2.5 kcal/mol Å². In order to evaluate the behavior of several guess collective variables to include in the final string pathway, several additional collective variables were calculated and recorded, but not

restrained. These include the z -component of the center of mass distance from the molecule to the upper leaflet phosphorus, the same distance for the lower leaflet phosphorus, the coordination number between the upper, lower, and both leaflet phosphorus and the permeant (as three independent collective variables), and lastly the coordination number between all water molecules and the permeant. It was determined that the inherently symmetric collective variables, which return approximately the same value at $\pm z$, are not informative for the string pathway. These include the coordination number of total phosphorus and the coordination number of water. While the coordination number of water coming from side a or side b of the membrane may be informative, water molecules are free to diffuse from side a to b across the membrane or trivially across the PBC. Therefore, labeling side a and b waters is highly impractical. The upper and lower leaflet phosphorus coordination numbers are particularly informative and practically implemented as collective variables because lipids generally do not switch leaflets on the timescale of a simulation. The collective variable of the z -component of the center of charge to center of mass distance was included post hoc as a way of restraining the molecule's orientation in a straightforward way.

Beginning with images from the steered-MD simulations, the mean force string method proceeded with first a 10 ns equilibration step with a 2.5 kcal/mol $\text{\AA}^2$ restraint on z only to estimate the initial minima of the other CVs. The optimization of the string proceeded for 5 more iterations. Because the CVs each have various units and change over different ranges, it was necessary to give each unique force constants. The force constant set to 2.45 kcal/mol $\text{\AA}^2$ for z , 49 kcal/mol units² for σ_{up} and σ_{low} , and 74.5 kcal/mol $\text{\AA}^2$ for Δz_H . Each step of the optimization was 10 ns, preceded by a 1 ns equilibrium during which the force constants were doubled. During string re-parameterization, it was necessary to normalize each CV so that its entire range is unity, which was done by dividing each by the respective maximum minus the minimum.

7.3.2 *PMF calculations*

The PMFs of three protonation states of nicotine across a POPC membrane along the z -coordinate were computed in our previous work using the WTM-eABF enhanced sampling method.²⁶ Here, we find a new use for the PMF of the singly protonated nicotine and POPC system.

The PMF of the auxiliary CV, ξ , was computed using umbrella sampling. The main reaction coordinate, z , was held stiffly at $z = 0$ with harmonic restraints for all of the windows with a force constant of 15 kcal/mol $\text{\AA}^2$. The centers of each window in terms of ξ were spaced by 0.1 from -3.0 to +3.0 units. The force constant was set to 400 kcal/mol based on trial and error to obtain overlap between the histograms of neighboring windows. The initial configurations for each window were obtained from the trajectories generated by the final iteration of the mean force string optimization, with ten initial configurations taken from each of the images from 30 to 40 (the center of region of the pathway). Each window was simulated for 100 ns, where the first 10 ns were considered equilibration and not included in the analysis. The PMF was computed from the sampling of ξ in each window using WHAM.¹¹⁰

7.3.3 *Short unbiased trajectories for transmission coefficient permeability calculation*

In order to compute the conditional average transmission coefficient permeability (eq 7.4, eq 7.6), a set of 100 short-unbiased trajectories of 500 ps in duration were generated starting from each of the discretized umbrella windows along ξ . The initial configurations were extracted from unique snapshots taken every 1 ns from each 100 ns umbrella window trajectory. Thermal equilibration was achieved by running each initial configuration for 100 ps with the restraints of the original umbrella window turned on. A total of 2850 ns of short-unbiased trajectories were generated for estimating the conditional transmission coefficient

permeability.

7.4 Results and discussion

7.4.1 *Re-weighted transmission coefficient permeability*

In previous work, we estimated the permeability of protonated nicotine based on the PMF computed with WTM-eABF enhanced sampling simulations and the position-dependent diffusivity computed with the mean-square deviation (MSD) in displacements.²⁶ The ISD model relates the PMF and position-dependent diffusivity to the permeability via the closed-form expression first derived by Diamond and Katz,⁸

$$P = \left[\int_{-L/2}^{L/2} dz \frac{e^{W(z)/k_B T}}{D(z)} \right]^{-1} \quad (7.7)$$

where $W(z)$ is the PMF, $D(z)$ is the position-dependent diffusivity, and L is the thickness of the membrane. When applying the ISD model, one must assume that the dynamics along the reaction coordinate z are overdamped and memoryless. While this assumption may be reasonable for neutral small molecules,¹³⁶ it is not expected to hold for situations in which there are slow orthogonal degrees of freedom, such as for ions and charged molecules.^{96;59}

Drawing on the findings of the steered-MD simulation and the optimized pathway with the mean force string method (Figure 7.1), it is apparent that there are several additional CVs that should be taken into consideration when calculating the permeability. In particular, it is clear that there is both a tradeoff in coordination number from lower to upper leaflet headgroups and a change in orientation of the nicotine molecule. We expect that this change in coordination numbers occurs in tandem with the change in orientation of the molecule. Physically, we understand the process at the center of the membrane to occur via a reorientation of the molecule such that the acidic proton, while initially pointing toward the lower leaflet headgroups, eventually points toward the upper leaflet headgroups. Around

the center images of the string, where the headgroup exchange and reorientation occurs, the CV z changes relatively slowly. This tapering of z is evidence that the permeation process is hindered by a barrier associated with this exchange of the center of charge between leaflets.

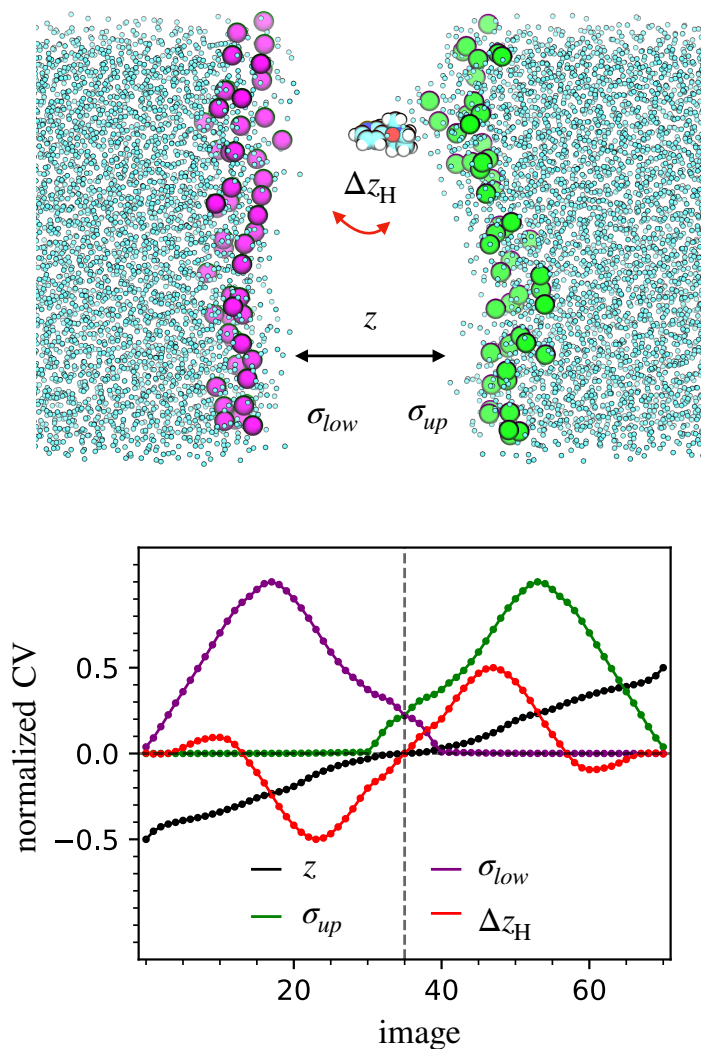


Figure 7.1: Illustration of the 4 CVs used for optimizing the pathway with the mean force string method and the converged string. Top: The phosphorus atoms of the lower leaflet are purple, while those of the upper leaflet are green. The acidic proton of nicotine is shown in red. For clarity, the water molecules are shown in cyan, and the lipid tails are hidden. Bottom: The string pathway which was optimized with the mean force string method. For visualization purposes, each CV is normalized individually such that the range is unity.

To simplify the physical picture and to obtain an accurate rate of barrier crossing in a straightforward way, we effectively wrapped the leaflet exchange process into a single

auxiliary CV, ξ , which is defined by difference between the two coordination numbers of the string pathway, $\sigma_{low} - \sigma_{up}$. To estimate the energetic cost of exchanging leaflets, we computed the PMF along ξ while z was harmonically restrained to 0. The PMFs of the primary and auxiliary CVs are contained in Figure 7.2. While the PMF along z has a large barrier height of around 12.2 kcal/mol, it alone does not control the rate for the permeation process. The PMF along ξ contains two wells that are separated by a barrier of 4.0 kcal/mol. It is also interesting to consider that even though the molecule is harmonically restrained to $z = 0$, the local minima are located at $\xi = \pm 1.23$. This finding indicates that the permeant prefers to be coordinated to either the upper or lower headgroups, even at the center of the membrane, and shared coordination between leaflets is highly disfavored.

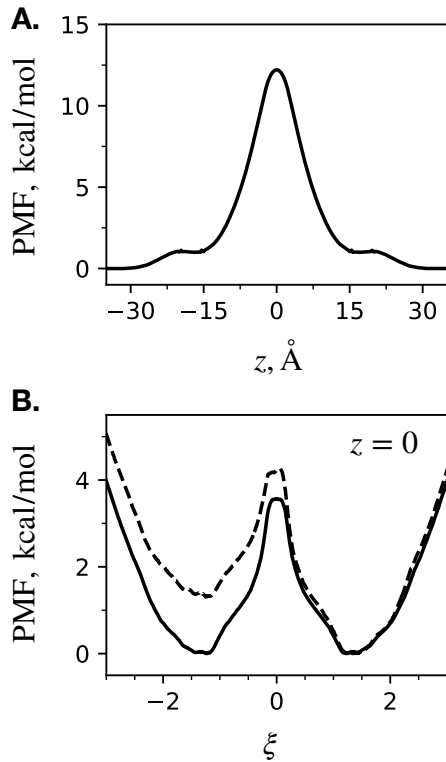


Figure 7.2: PMFs of the primary and auxiliary CVs of protonated nicotine across the POPC membrane. **A.** The PMF along z , the primary CV for the permeation process. **B.** The PMF along ξ , which was computed with umbrella sampling, while the value of z was stiffly harmonically restrained to be 0. The dashed line is the raw PMF, and the solid line is symmetrized about $\xi = 0$.

Computing the PMF of ξ with z restrained to 0 allowed for extensive sampling of the

center of the membrane. In standard umbrella sampling along z , for example, the permeant would most likely only sample a single well in the PMF of ξ . By deliberately sampling along ξ , it is possible to generate ensembles of coordinates from that would otherwise be inaccessible on typical simulation timescales. It was shown by Hinsen and Roux²²⁹ that the transmission coefficient⁶⁶ can be estimated more efficiently by re-weighting contributions along an auxiliary CV acting as a gating mode on the reaction. The re-weighting procedure is defined by eq 7.4 in the case of the transmission permeability and eq 7.6 in the case of the indicator-restricted committor transmission permeability. If the naive approach is taken, the transmission permeability can be estimated by initiating a large number of short unbiased trajectories, with initial coordinates generated from an umbrella sampling simulation at $z = 0$. Intuitively, for a permeant such as protonated nicotine, all of the initial coordinates would be coordinated to a single leaflet, and each short unbiased trajectory would deterministically fall back to the corresponding side of the membrane. The transmission coefficient estimated from these trajectories would not be physically meaningful. To avoid this complication, we generated the initial coordinates for the short unbiased trajectories from each of the ξ umbrella sampling windows. In this way, both wells of ξ and even the low probability $\xi = 0$ will be sampled. Because the resulting ensemble of trajectories will not correspond to the equilibrium probability at $z = 0$, it is necessary to apply the reweighting scheme of eq 7.6 and eq 7.4.

An illustration of the initial and final 2-dimensional coordinates of the short unbiased trajectories in CV space are shown in Figure 7.3. It is clear from the coloring scheme based on the initial value of ξ that the fate of each trajectory is strongly correlated with leaflet coordination. This result also demonstrates that by taking initial coordinates from umbrella sampling along the auxiliary variable, both sides of the membrane will be sampling approximately evenly. From a total of 5700 trajectories (100 from each umbrella window excluding the first and last windows), 2146 ended with $z < -5 \text{ \AA}$, 2096 ended with $z > 5$

$\text{\AA}$, and 1458 ended inside of this range. Notably, the peak values of σ_{low} and σ_{up} in the optimized string pathway correspond to coordination numbers of 3.2. These maxima in coordination occur in the images for which $z = \pm 16.3 \text{ \AA}$.

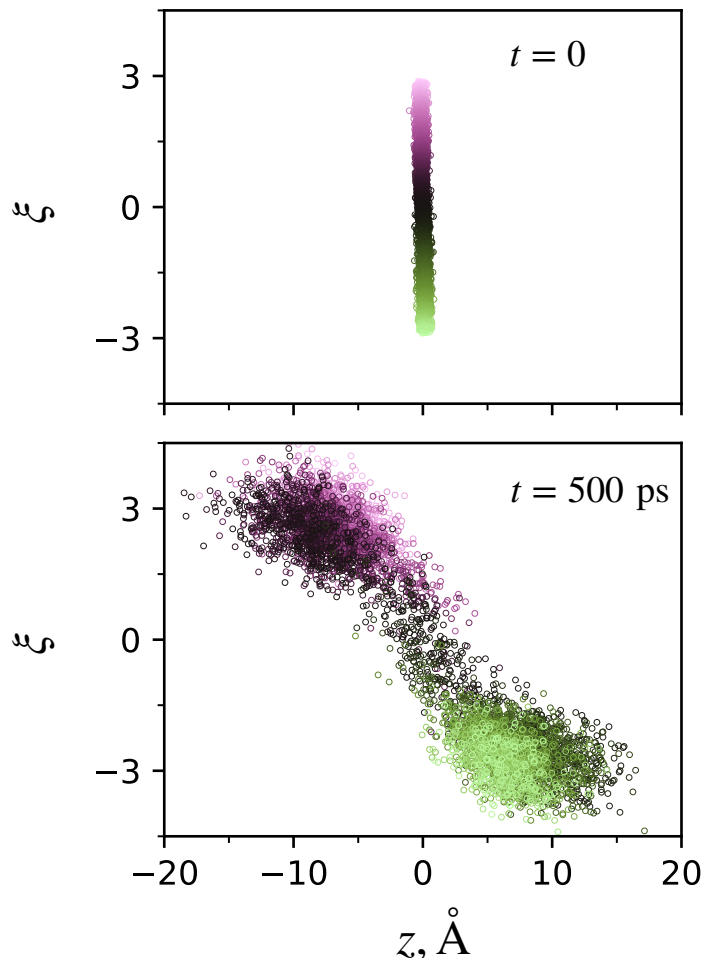


Figure 7.3: Scatter plots of the 2-dimensional coordinates for each short unbiased trajectory for $t = 0$ (top) and $t = 500$ (bottom) ps. The color of each point at $t = 500$ is based on its value of ξ at $t = 0$. There are a total of 5700 trajectories, for a total of 2850 ns of sampling.

The permeability computed from the set of short unbiased trajectories is shown in Figure 7.4. The permeability was estimated via eq 7.4 for the transmission coefficient with re-weighting along ξ , and also eq 7.6, which relies on the same approach, except in terms of the committor probability. In general, introducing the committor into the expression for the transmission coefficient greatly reduces the noise and oscillations for the range of lag

times that was considered. This observation is in line with our previous findings regarding the committor time-correlation function, which was shown to reach Markovian lag time faster than the time-correlation function of purely indicator functions.^{13;136} For reference, the ISD permeability is also displayed in Figure 7.4, which was calculated in the previous paper using the MSD diffusivity and the PMF along z only. Notably, the converged committor permeability is in reasonable agreement with the ISD permeability. Because the ISD model, in theory, does not account at all for the additional 4 kcal/mol barrier of exchanging leaflet coordination, we further investigated why the two estimates are so similar. A possible explanation is that the MSD diffusivity is impacted by the orthogonal degree of freedom at the center of the membrane. In order to estimate the MSD diffusivity, simulations were run using the converged ABF forces, effectively allowing for free diffusion across the entire membrane. The MSD diffusivity decreases, however, as the lag time increases, indicating dynamics that is not strictly diffusive. It is possible that the MSD diffusivity in the center of the membrane decreases similar to a Brownian particle diffusing near a wall.

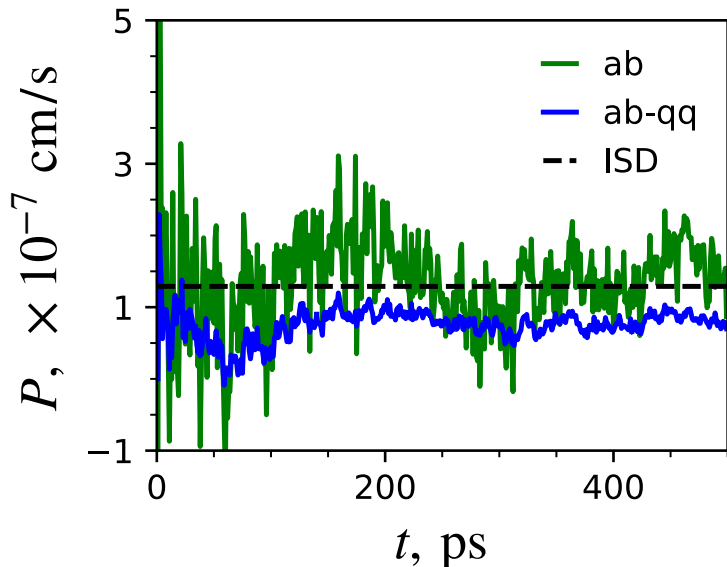


Figure 7.4: The permeability of protonated nicotine based on the re-weighted transmission coefficient of eq 7.4 (green line), the re-weighted indicator restricted committor transmission coefficient of eq 7.6 (blue line), and the ISD model of eq 7.7 (dashed black line).

7.5 Concluding discussion

We have shown that the permeability of protonated nicotine based on the reactive flux formulation is in good agreement with the ISD permeability. The reactive flux permeability was computed from short unbiased trajectories that were initiated at the top of the barrier. For additional sampling, the short unbiased trajectories were weighted according to their equilibrium probability with respect to a significant orthogonal degree of freedom. The ISD permeability was computed from the PMF and position-dependent diffusivity, which were extracted from enhanced sampling WTM-eABF simulations in our previous work. Work remains to be done to suggest a reason why the ISD permeability is approximately correct, despite the presence of a 4 kcal/mol barrier in an orthogonal degree of freedom at the top of the PMF along z .

CHAPTER 8

CONCLUSION

It is worth noting the recent developments in the field of membrane permeation. In particular, several advances have been made since the beginning of the projects described here. The exact non-Markovian permeability can be determined with transition interface sampling, even for a molecule with a significant barrier across the membrane.²³⁰ In the field of peptide permeation,²³¹ it was shown that Milestoning can be used to account for significant membrane defects.⁶⁰ It is possible that the future of permeability, in the context of drug design, lies in automation.^{150;151;226} In a recent study, Ma et al.²²⁶ first constructed a dataset of thousands of small molecule PMFs across a membrane with umbrella sampling coarse-grained molecular dynamics simulations. The relative permeability was assessed based on the ISD model and PMFs. They then used the dataset to train a graph neural network for predicting and optimizing the permeability of new molecules. The questions remains, however, whether the ISD model is valid for the range of molecules that were considered. It is expected that such an automatic screening process for peptide permeation would be significantly more difficult due to membrane deformation and additional slow degrees of freedom.

Experimentally, the permeation of the three protonation states of nicotine remains relevant.^{227;232} As new methods of nicotine intake become popularized, the effects of the initial form of the nicotine molecules are often closely related to each protonation state’s intrinsic permeability. For instance, when smoked, nicotine enters the body at a low pH, while vapes are generally lower pH.²²⁷ Alternatively, nicotine pouches, through which the nicotine absorbs into the mouth lining, raises the pH of the mouth over the time period that they are used.²³² The pH-partition hypothesis, which states that ionization states are fixed, is a critical assumption for studies which claim that the permeability of nicotine depends on its initial pH when it enters the body.

A final consideration is the accuracy of permeability calculations from molecular dynamics simulations, which are ultimately limited by the force field itself. An unanswered question in this area is why the Drude2023 force field has excellent agreement with experimental water permeability for saturated, but not for unsaturated phosphocholine membranes.^{30;29} The day may be approaching when a minimum membrane (roughly 16 lipids) can be simulated with a quantum mechanical representation. Early efforts have already been made, such as by Mallick et al.²³³ While it is expected that larger membranes and much longer simulation times would be needed than those conducted by Mallick et al., it will likely be possible to resolve force field confusion with density functional theory in the not too distant future.

APPENDIX A

SUPPORTING INFORMATION FOR CHAPTER 2

A.1 Derivation of analytical MSD in overdamped GLE with exponential memory and harmonic potential

The generalized Langevin equation (GLE) is,^{71;14;72}

$$m\ddot{z} = F(z) - \int_0^t M(t') \dot{z}(t-t') dt' + f(t) \quad (\text{A.1})$$

where $F(z) = -W'(z)$ is the mean force based on the PMF of the permeant molecule across the membrane, $M(t)$ is the memory function, and $f(t)$ is the random force obeying the fluctuation-dissipation theorem, $\langle f(t)f(0) \rangle = k_B T M(t)$. For a harmonic functional form, the potential is $W(z) = \frac{1}{2}(W'')z^2$, and the mean force is $F = -W'' z$. For a harmonic well, $W'' > 0$, whereas $W'' < 0$ for an inverted harmonic barrier. Let us define the effective exponential memory function,

$$M(t) = \delta(t)\gamma_z + K_e e^{-t/\tau_e}$$

Where $\gamma_z = k_B T / D_z$, and $\tau_e = k_B T / (D_e K_e)$. According to Tuckerman and Berne,^{77;9}

$$\mathcal{L}[\ddot{z}] = -v_0 + s(-z_0 + s\mathcal{L}[z]) \quad (\text{A.2})$$

where $\mathcal{L}[z] = \tilde{z}(s)$ denotes the Laplace transform of $z(t)$. We solve for the position in Laplace space,

$$\tilde{z}(s) = z_0 \tilde{C}_{11}(s) + v_0 \tilde{C}_{12}(s) + \frac{\tilde{f}(s)/m}{W''/m + s^2 + s\tilde{M}(s)/m} \quad (\text{A.3})$$

where,

$$\tilde{C}_{11}(s) = \frac{s + \tilde{M}(s)/m}{W''/m + s^2 + s\tilde{M}(s)/m} \quad (\text{A.4})$$

$$\tilde{C}_{12}(s) = \frac{1}{W''/m + s^2 + s\tilde{M}(s)/m} \quad (\text{A.5})$$

In the time domain, the position and variance are given by,

$$z(t) = z_0 C_{11}(t) + v_0 C_{12}(t) + \frac{1}{m} \int_0^t dt' C_{12}(t-t') f(t') \quad (\text{A.6})$$

$$\langle z^2(t) \rangle - \langle z(t) \rangle^2 = -\frac{k_B T}{W''} ((C_{11}(t))^2 - 1) - \frac{k_B T}{m} (C_{12}(t))^2 \quad (\text{A.7})$$

where $\mathcal{L}^{-1}[\tilde{C}(s)] = C(t)$ is the inverse Laplace transform. In the overdamped limit in which $m \rightarrow 0$,

$$\lim_{m \rightarrow 0} C_{11}(s) = \frac{\tilde{M}(s)}{W'' + s\tilde{M}(s)} \quad (\text{A.8})$$

$$\lim_{m \rightarrow 0} C_{12}(s) = 0 \quad (\text{A.9})$$

The Laplace transform of the memory function is determined analytically,

$$\tilde{M}(s) = \int_0^\infty dt M(t) e^{-st} \quad (\text{A.10})$$

$$\tilde{M}(s) = \gamma_z + \frac{K_e}{s + \frac{1}{\tau_e}} \quad (\text{A.11})$$

We insert $\tilde{M}(s)$ into the expression for the overdamped $\tilde{C}_{11}(s)$ to obtain the explicit form,

$$\tilde{C}_{11}(s) = \frac{\gamma_z + \frac{K_e}{s + \frac{1}{\tau_e}}}{W'' + s\left(\gamma_z + \frac{K_e}{s + \frac{1}{\tau_e}}\right)} \quad (\text{A.12})$$

$$\tilde{C}_{11}(s) = \frac{\gamma_z s + \gamma_z/\tau_e + K_e}{W''s + W''/\tau_e + s^2\gamma_z + s\gamma_z/\tau_e + sK_e} \quad (\text{A.13})$$

$$\tilde{C}_{11}(s) = \frac{\gamma_z s + \gamma_z/\tau_e + K_e}{s^2\gamma_z + s(\gamma_z/\tau_e + K_e + W'') + W''/\tau_e} \quad (\text{A.14})$$

$$\tilde{C}_{11}(s) = \frac{s + 1/\tau_e + K_e/\gamma_z}{s^2 + (s/\gamma_z)(\gamma_z/\tau_e + K_e + W'') + W''/\gamma_z\tau_e} \quad (\text{A.15})$$

The variance in $z(t)$ is then easily obtained analytically once an explicit expression for $C_{11}(t)$ is determined from the inverse Laplace transform of $\tilde{C}_{11}(s)$. We begin with partial fraction decomposition and then recognize the result as the Laplace transform of a sum of exponential functions. We factor the denominator by solving for s ,

$$s^2 + (s/\gamma_z)(\gamma_z/\tau_e + K_e + W'') + W''/\gamma_z\tau_e = 0 \quad (\text{A.16})$$

where the solutions are,

$$\begin{aligned} s_{\pm} &= \frac{1}{2} \left(- (1/\gamma_z)(\gamma_z/\tau_e + K_e + W'') \pm \sqrt{(1/\gamma_z)^2(\gamma_z/\tau_e + K_e + W'')^2 - 4W''/\gamma_z\tau_e} \right) \\ s_{\pm} &= \frac{1}{2\gamma_z\tau_e} \left(- (\gamma_z + K_e\tau_e + W''\tau_e) \pm \sqrt{(\gamma_z + K_e\tau_e + W''\tau_e)^2 - 4W''\gamma_z\tau_e} \right) \end{aligned} \quad (\text{A.17})$$

or,

$$s_+ = \frac{1}{2\gamma_z\tau_e} \left(- (\gamma_z + K_e\tau_e + W''\tau_e) + \sqrt{(\gamma_z + K_e\tau_e + W''\tau_e)^2 - 4W''\gamma_z\tau_e} \right) \quad (\text{A.18})$$

$$s_- = \frac{1}{2\gamma_z\tau_e} \left(- (\gamma_z + K_e\tau_e + W''\tau_e) - \sqrt{(\gamma_z + K_e\tau_e + W''\tau_e)^2 - 4W''\gamma_z\tau_e} \right) \quad (\text{A.19})$$

With the factored denominator, eq A.15 simplifies to,

$$\tilde{C}_{11}(s) = \frac{s + 1/\tau_e + K_e/\gamma z}{(s - s_+)(s - s_-)} \quad (\text{A.20})$$

which can be expressed as the sum of fractions,

$$\tilde{C}_{11}(s) = \frac{P_1}{(s - s_+)} + \frac{P_2}{(s - s_-)} \quad (\text{A.21})$$

Where P_1 and P_2 are constant with respect to s because the numerator of the original expression is linear in s and the denominator is quadratic in s . We recognize the exact inverse Laplace transform of the above form as,

$$\mathcal{L}^{-1}[\tilde{C}_{11}(s)] = P_1 e^{ts_+} + P_2 e^{ts_-} \quad (\text{A.22})$$

Solving for P_1 and P_2 requires some algebra,

$$\frac{s + 1/\tau_e + K_e/\gamma z}{(s - s_+)(s - s_-)} = \frac{P_1}{(s - s_+)} + \frac{P_2}{(s - s_-)} \quad (\text{A.23})$$

$$\frac{s + 1/\tau_e + K_e/\gamma z}{(s - s_+)(s - s_-)} = \frac{P_1 s - P_1 s_- + P_2 s - P_2 s_+}{(s - s_+)(s - s_-)} \quad (\text{A.24})$$

$$P_1 s + P_2 s = s \quad (\text{A.25})$$

$$P_1 + P_2 = 1 \quad (\text{A.26})$$

$$-P_1 s_- - P_2 s_+ = 1/\tau_e + K_e/\gamma z \quad (\text{A.27})$$

$$-(1 - P_2)s_- - P_2 s_+ = 1/\tau_e + K_e/\gamma z \quad (\text{A.28})$$

$$P_2(s_- - s_+) - s_- = 1/\tau_e + K_e/\gamma z \quad (\text{A.29})$$

$$P_2 = \frac{1}{s_- - s_+} (s_- + 1/\tau_e + K_e/\gamma z) \quad (\text{A.30})$$

$$P_1 = 1 - \frac{1}{s_- - s_+} (s_- + 1/\tau_e + K_e/\gamma z) \quad (\text{A.31})$$

We can express $C_{11}(t)$ explicitly as,

$$\begin{aligned} C_{11}(t) &= \left(1 - \frac{1}{s_- - s_+} (s_- + 1/\tau_e + K_e/\gamma_z)\right) e^{ts_+} \\ &+ \left(\frac{1}{s_- - s_+} (s_- + 1/\tau_e + K_e/\gamma_z)\right) e^{ts_-} \end{aligned} \quad (\text{A.32})$$

We can better show the explicit dependence on the parameters of the memory function with some rearrangements,

$$\begin{aligned} C_{11}(t) &= \frac{1}{s_- - s_+} \left[\left((s_- - s_+) - s_- - 1/\tau_e - K_e/\gamma_z \right) e^{ts_+} \right. \\ &\quad \left. + \left(s_- + 1/\tau_e + K_e/\gamma_z \right) e^{ts_-} \right] \end{aligned} \quad (\text{A.33})$$

$$\begin{aligned} C_{11}(t) &= \frac{1}{s_- - s_+} \left[\left(-s_+ - 1/\tau_e - K_e/\gamma_z \right) e^{ts_+} \right. \\ &\quad \left. + \left(s_- + 1/\tau_e + K_e/\gamma_z \right) e^{ts_-} \right] \end{aligned} \quad (\text{A.34})$$

Define, $B = \frac{1}{2\gamma_z\tau_e} \sqrt{(\gamma_z + K_e\tau_e + W''\tau_e)^2 - 4W''\gamma_z\tau_e}$

$$s_+ = -\frac{1}{2\tau_e} - \frac{K_e}{2\gamma_z} - \frac{W''}{2\gamma_z} + B \quad (\text{A.35})$$

$$s_- = -\frac{1}{2\tau_e} - \frac{K_e}{2\gamma_z} - \frac{W''}{2\gamma_z} - B \quad (\text{A.36})$$

Insert s_+ and s_- ,

$$\begin{aligned} C_{11}(t) &= \frac{1}{-2B} \left[\left(-B + \frac{1}{2\tau_e} + \frac{K_e}{2\gamma_z} + \frac{W''}{2\gamma_z} - 1/\tau_e - K_e/\gamma_z \right) e^{ts_+} \right. \\ &\quad \left. + \left(-B - \frac{1}{2\tau_e} - \frac{K_e}{2\gamma_z} - \frac{W''}{2\gamma_z} + 1/\tau_e + K_e/\gamma_z \right) e^{ts_-} \right] \end{aligned} \quad (\text{A.37})$$

$$\begin{aligned} C_{11}(t) &= \frac{1}{-2B} \left[\left(-B - \frac{1}{2\tau_e} - \frac{K_e}{2\gamma_z} + \frac{W''}{2\gamma_z} \right) e^{ts_+} \right. \\ &\quad \left. + \left(-B + \frac{1}{2\tau_e} + \frac{K_e}{2\gamma_z} - \frac{W''}{2\gamma_z} \right) e^{ts_-} \right] \end{aligned} \quad (\text{A.38})$$

$$\begin{aligned} C_{11}(t) &= \frac{1}{2B} \left[\left(B + \frac{1}{2\tau_e} + \frac{K_e}{2\gamma_z} - \frac{W''}{2\gamma_z} \right) e^{ts_+} \right. \\ &\quad \left. + \left(B - \frac{1}{2\tau_e} - \frac{K_e}{2\gamma_z} + \frac{W''}{2\gamma_z} \right) e^{ts_-} \right] \end{aligned} \quad (\text{A.39})$$

A.2 Derivation of Grote-Hynes correction to ISD permeability

Starting from the general form of Grote-Hynes theory,¹⁶

$$\omega_0^2 - s^2 = \frac{s}{m} \widetilde{M}(s) \quad (\text{A.40})$$

where $\omega_0^2 = -W''/m$ ($W'' < 0$ for an inverted barrier). Neglecting the inertial term ms^2 and taking the overdamped limit as m approaches 0,

$$\omega_0^2 m \approx s \widetilde{M}(s) \quad (\text{A.41})$$

$$-W'' \approx s \widetilde{M}(s) \quad (\text{A.42})$$

The Laplace transform of the exponential memory function is,

$$\widetilde{M}(s) = \gamma_z + \frac{K_e}{s + 1/\tau_e} \quad (\text{A.43})$$

After substituting $\widetilde{M}(s)$ and W'' into eq A.42,

$$s \left(\gamma_z + \frac{K_e}{s + 1/\tau_e} \right) + W'' = 0 \quad (\text{A.44})$$

$$s\gamma_z(s + 1/\tau_e) + sK_e + W''(s + 1/\tau_e) = 0 \quad (\text{A.45})$$

$$s^2\gamma_z + \frac{s\gamma_z}{\tau_e} + sK_e + sW'' + \frac{W''}{\tau_e} = 0 \quad (\text{A.46})$$

$$s^2\gamma_z\tau_e + s \left(\gamma_z + K_e\tau_e + W''\tau_e \right) + W'' = 0 \quad (\text{A.47})$$

The solutions are the same as eq A.17. Here we consider the positive root,

$$s_+ = \frac{1}{2\gamma_z\tau_e} \left(- \left(\gamma_z + K_e\tau_e + W''\tau_e \right) + \sqrt{\left(\gamma_z + K_e\tau_e + W''\tau_e \right)^2 - 4W''\gamma_z\tau_e} \right) \quad (\text{A.48})$$

We determine the approximate solution by neglecting the s^2 term in eq A.47

$$s \approx \frac{-W''}{(\gamma_z + K_e\tau_e + W''\tau_e)} \quad (\text{A.49})$$

$$s \approx \left(- \frac{W''}{\gamma_z + K_e\tau_e} \right) \left(\frac{1}{1 + \frac{W''\tau_e}{\gamma_z + K_e\tau_e}} \right) \quad (\text{A.50})$$

The effective diffusivity in terms of the memory function parameters is,

$$D_{\text{eff}} = \frac{k_B T}{\gamma_{\text{tot}}} \quad (\text{A.51})$$

$$D_{\text{eff}} = \frac{k_B T}{\gamma_z + K_e\tau_e} \quad (\text{A.52})$$

The transmission coefficient is then,

$$\kappa = \sqrt{-mW''} \frac{D_{\text{eff}}}{k_B T} \left(\frac{1}{1 + W''D_{\text{eff}}\tau_e/k_B T} \right) \quad (\text{A.53})$$

Which corresponds to the permeability coefficient,

$$P = P_{\text{ISD}} \left(\frac{1}{1 + W'' D_{\text{eff}} \tau_e / k_B T} \right) \quad (\text{A.54})$$

$$= P_{\text{ISD}} \left(\frac{1}{1 - \tau_e / \tau_0} \right) \quad (\text{A.55})$$

where $\tau_0 = k_B T / |W''| D_{\text{eff}}$.

A.3 Heaviside correlation function permeability from BD simulations with and without memory.

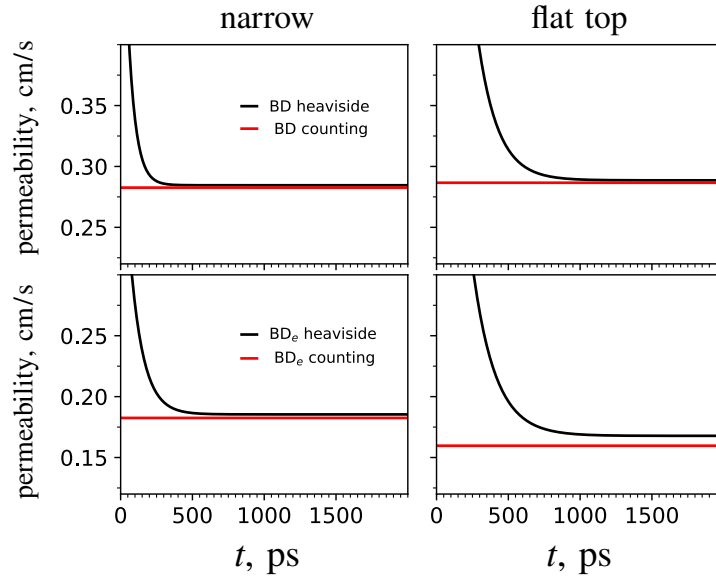


Figure A.1: Permeability computed with the Heaviside correlation function (black line) for BD (top) and BD_e (bottom) simulations of the narrow (left) and flat top (right) barriers. The corresponding counting permeability are shown for reference. The heaviside permeability plateau values at 2000 ps and 3000 ps for the BD_e narrow and flat top systems are 0.185 and 0.162 cm/s, respectively.

A.4 Position-dependent diffusivity of water in DLPC from umbrella sampling.

In order to estimate the ISD permeability of DLPC to water, we computed the position-dependent diffusivity with umbrella sampling (US). The diffusivity in a harmonic well is determined from,^{84;5}

$$D(z) = \frac{\text{var}^2(z)}{\int_0^\infty C_{zz}(t)dt} \quad (\text{A.56})$$

where $C_{zz}(t) = \langle \delta z(t) \delta z(0) \rangle$. The overlap in the sampling of the 1 Å spaced windows is shown in the probability density profiles in Figure A.2. We evaluate the integral over $C_{zz}(t)$ with $dt = 1$ fs. To investigate the effect of the upper limit of integration of $C_{zz}(t)$ in the

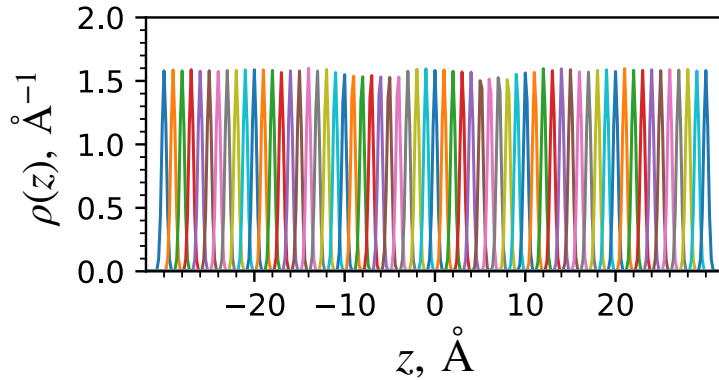


Figure A.2: Umbrella window probability density for water in DLPC with a harmonic force constant of 10 kcal/mol Å² and centers spaced at 1 Å intervals.

denominator of eq A.56 on the position-dependent diffusivity profile, we plot the resulting $D(z)$ for a range of upper limits in Figure A.3. The diffusivity profile seems to have largely converged between 100 to 400 ps. Notably, the diffusivity in the bulk water region for all of the shown upper limits is close to the known value of SWM4 diffusivity of 0.23 Å²/ps.⁸⁰. We also note that the diffusivity profile for 5 ps is very similar to that determined by Riahi et al.²³⁴ with US for SWM4 water in a DPPC membrane represented by the Drude force field, which was also integrated with a reported upper limit of 5 ps. For our 400 ps profile,

there are strange artifacts near the interface of water and the membrane. We therefore implemented a method to eliminate noise from the calculation of the integral of $C_{zz}(t)$ following a similar method used by Dickson et al.,³⁸ however with a stricter cutoff condition. The upper limit of the integral is determined separately for each window, according to the condition that $C_{zz}(t_\infty) < 10^{-5} \text{ var}(z)$ or else $t_\infty = 500$ ps. The resulting diffusivity profile and ISD permeability from this analysis are shown in Figure A.4A. The ISD permeability of 2.09×10^{-3} cm/s is close to the ISD permeability determined from the 400 ps profile, which is 2.00×10^{-3} cm/s. We show the integral of $C_{zz}(t)$ as a function of the upper limit of integration for several windows in Figure A.4B., where the automatically chosen t_∞ is marked on each line.

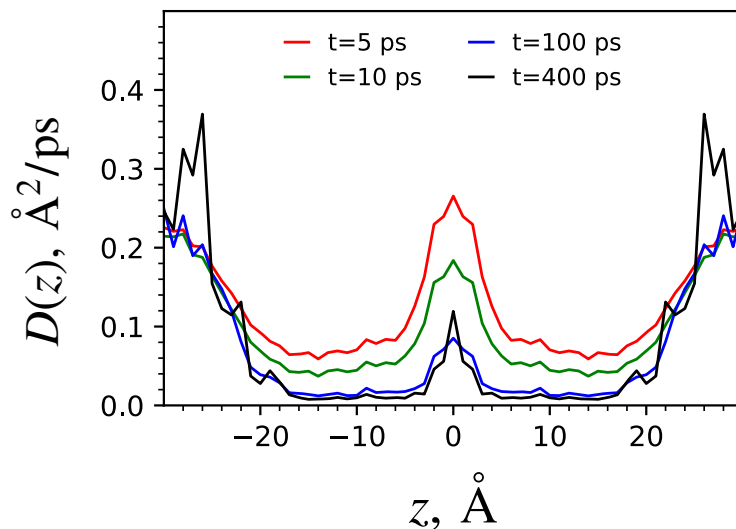


Figure A.3: Position-dependent diffusivity profile of water in DLPC, over a range of upper limits of the integral in eq A.56.

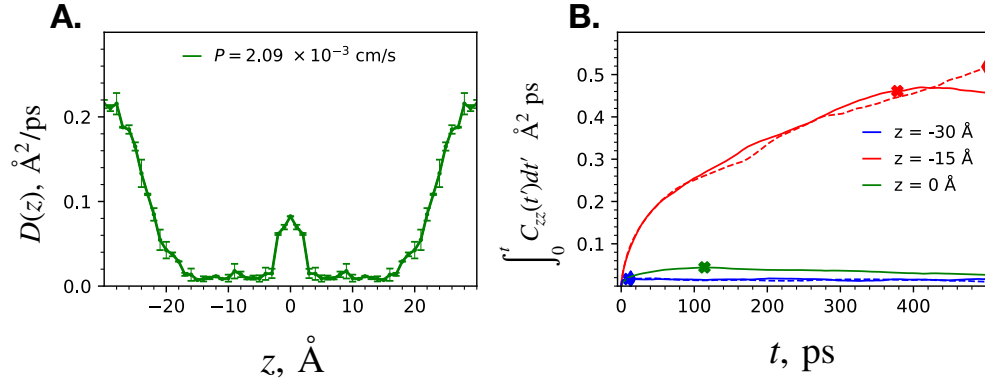


Figure A.4: **A.** Position-dependent diffusivity profile of water in DLPC, under the condition that the upper limit of the integral (t_∞) in eq A.56 satisfies $C_{zz}(t_\infty) < 10^{-5} \text{ var}(z)$ or else $t_\infty = 500$ ps. The reported permeability is determined with the $D(z)$ profile and the PMF according to the ISD model. The ISD model is integrated with a linearly interpolated $D(z)$ profile, from an original resolution of 1 Å to a final resolution of 0.2 Å. $D(z)$ is symmetrized, with error bars calculated by $\pm \frac{1}{2} |D(z) - D(-z)|$. **B.** Integral of $C_{zz}(t)$ as a function of the upper limit of integration for several windows in different regions of the membrane. The dashed lines indicate the corresponding positive window. The “X” and “diamond” markers indicate where the automatic cutoffs were placed for calculation of **A**.

APPENDIX B

SUPPORTING INFORMATION FOR CHAPTER 3

B.1 Statistical analysis of equilibrium and short unbiased 2D-Brownian dynamics trajectories

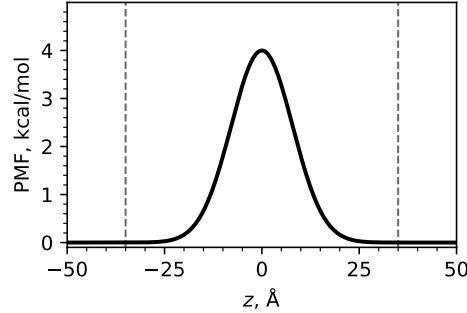


Figure B.1: The potential of mean force (PMF) associated with the 2-dimensional Brownian dynamics simulations, given analytically by a Gaussian function. The cutoff values for the permeability calculations, $\pm L/2$, are indicated with vertical dashed lines where $z = \pm 35$ Å. The hard reflecting walls of the simulation box are located where $z = \pm 50$ Å.

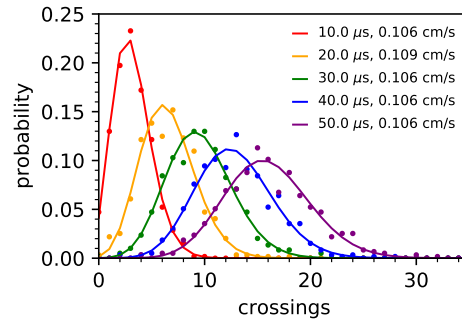


Figure B.2: Discrete probability distribution of membrane crossings, based on 593 independent 2-dimensional Brownian dynamics stochastic simulations, each $50 \mu s$ in duration. Each color corresponds to a Poisson interval, and the solid line represents the best fitting Poisson distribution. The permeability for each interval is the mean number of crossings divided by $2t\pi(-L/2)$, where t is the duration of the interval, and $\pi(-L/2)$ is the equilibrium spatial probability at the edge of the membrane.

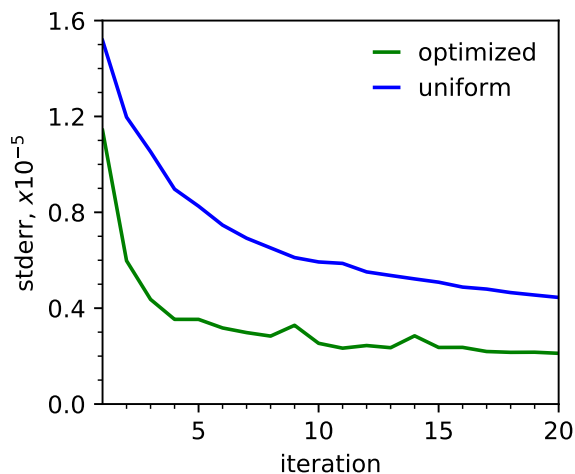


Figure B.3: Assessment of the automatic optimization of initial positions for short unbiased 2-dimensional Brownian dynamics stochastic simulations. The standard error (square root of the global variance) in the committor time-correlation function for $t^* = 300$ ps is calculated for each of the 20 iterations of optimized initial positions (green line), as well as for 20 iterations of strictly uniform initial positions (blue line). Each line represents the mean standard error of 50 independent sets of 20 iterations each.

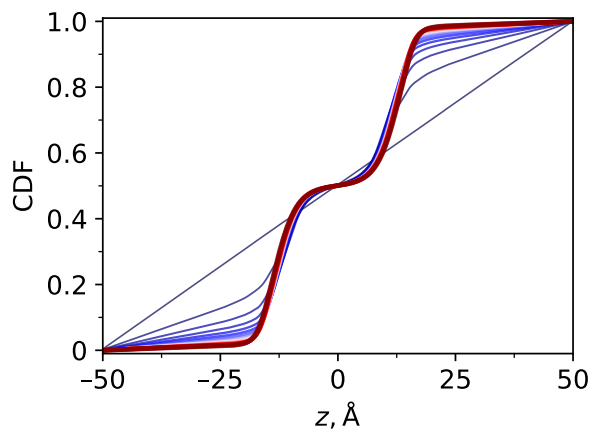


Figure B.4: Cumulative distribution function (CDF) of the initial positions obtained by the automatic optimization scheme for short unbiased 2D-Brownian dynamics stochastic simulations. Each of the 20 lines corresponds to an iteration of the optimization scheme, beginning with dark blue, diverging through white, and ending with dark red. Each CDF is constructed from the mean distribution of 50 replicate simulations for each iteration

B.2 Committor, distributions of initial positions, and error analysis of short unbiased MD simulations of sucrose permeation

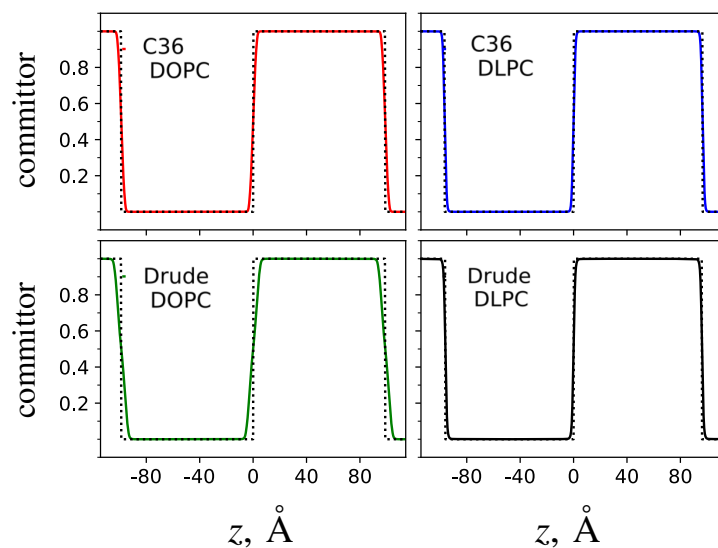


Figure B.5: Unwrapped committor probability for each sucrose and membrane system, which were extended to cover one additional periodic image in the positive and negative directions along z . The committor probabilities were computed using the approximate committor expression, where it was assumed that $D(z)$ does not depend on position. The black dotted lines correspond to the indicator function, $H_B(z)$.

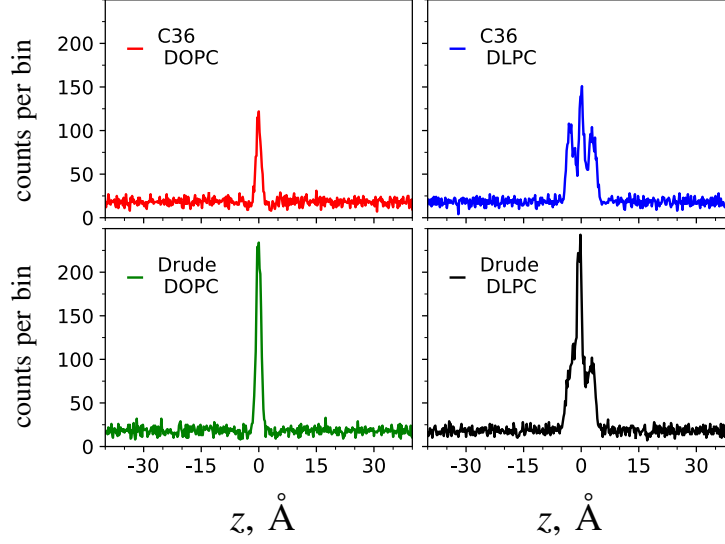


Figure B.6: Histogram of the initial z positions of each short unbiased trajectory used for re-weighting the time-correlation functions for each set of sucrose MD simulations. Each histogram corresponds to the weighting factor w_n within the re-weighting scheme. Each bin has a width of $0.2 \text{ \AA}$

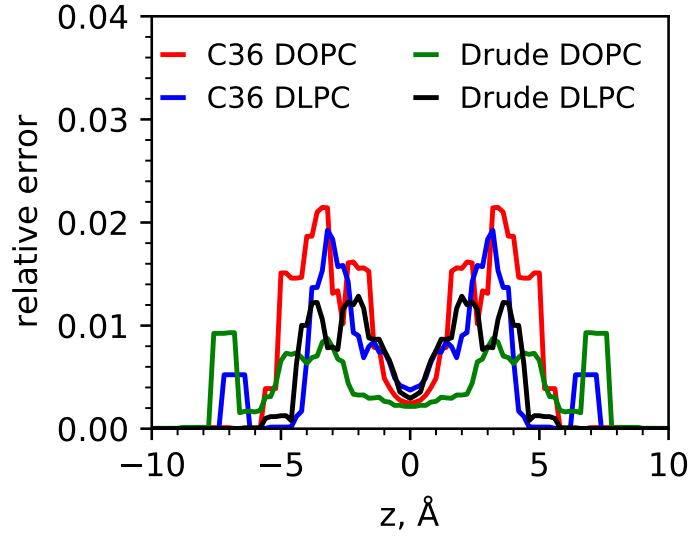


Figure B.7: Relative error of the committor time-correlation function within each z_n bin for each force field and membrane system of the sucrose MD simulations. The relative error is defined as the standard error in each bin divided by the value of the mean time-correlation function. The magnitude of the relative error indicates the impact that additional sampling in each z_n bin will have on decreasing the global variance.

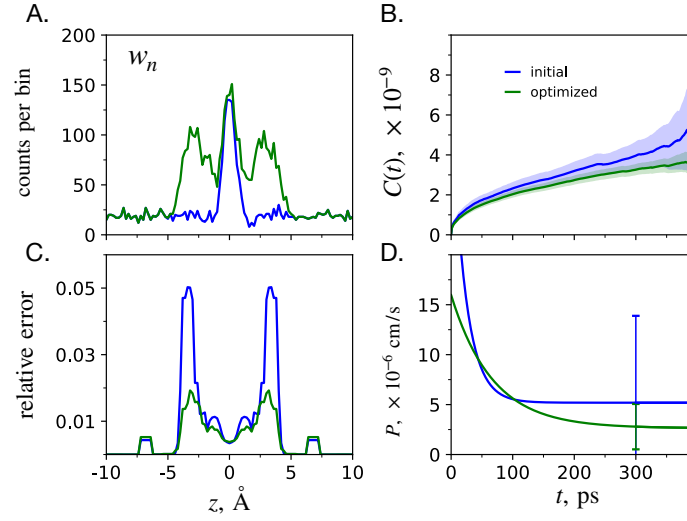


Figure B.8: Illustration of error analysis and optimization of the C36 DLPC system of sucrose permeation. In each case, the blue line represents the initial set of short unbiased trajectories, and the green line indicates the optimized set with additional sampling. **A.** Histogram of each set of initial positions, corresponding to the weighting factors w_n . **B.** Committor time-correlation function calculated with each set of trajectories. The errorbars correspond to $\pm$ stderr. **C. D.** Permeability based on the committor time-correlation function for each set of trajectories. The errorbars correspond to $\pm$ stderr in the plateau region.

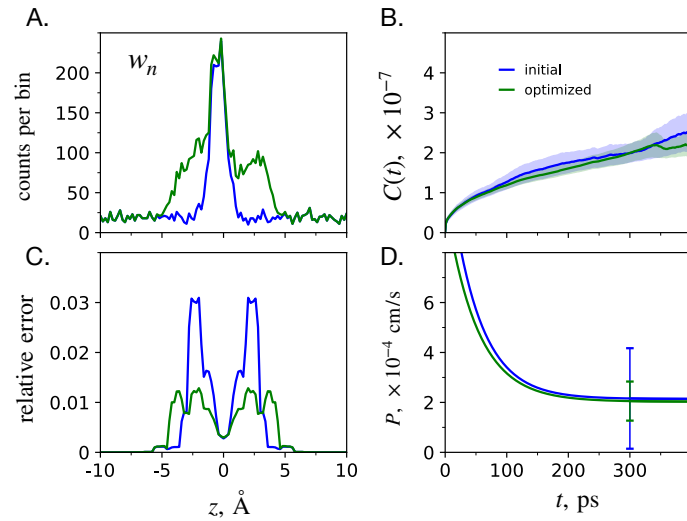


Figure B.9: Illustration of the same error analysis as Figure B.8 repeated for the Drude DLPC system.

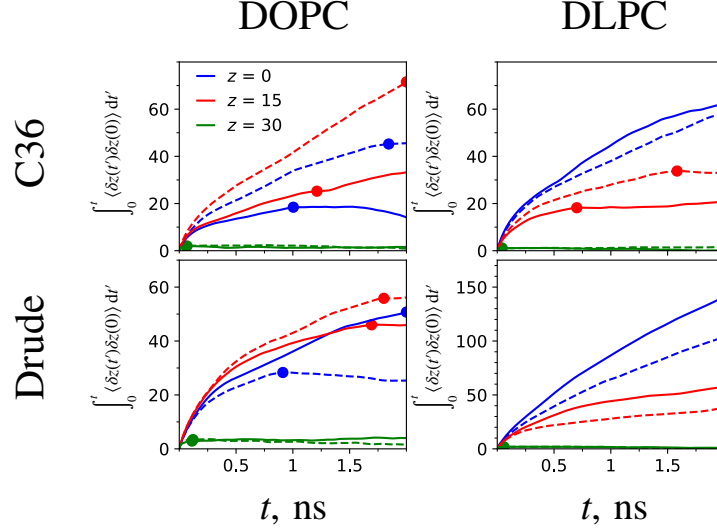


Figure B.10: Integral of the position autocorrelation function for each system for several windows. The dashed lines correspond to the negative z window. The dots indicate the value of the integral used for the calculation of the position-dependent diffusivity for each window, such that the value of the integral has increased by less than 0.01 times the variance in z , or else the value for $t = 2$ ns is used.

B.3 Position-dependent diffusivity

The position-dependent diffusivity of each sucrose system was computed with the method of Hummer.⁵ For each umbrella window, the local diffusivity corresponds to,

$$D(z) = \frac{\text{var}^2(z)}{\int_0^\infty \langle \delta z(t) \delta z(0) \rangle dt} \quad (\text{B.1})$$

We applied a condition to automatically determine the upper limit of integration for the denominator of eq B.1, similar to that used by others³⁸ and us previously.¹³ We chose the condition for the cutoff to be that the value of the integral has increased by less than 0.01 times the variance in z , or else the value for $t = 2$ ns is used. The values of the integral for a range of upper limits in different regions of the membrane are shown in Figure B.10. Using a dt of 1 ps, we considered upper limits up to 2 ns.

B.4 Hydration number of sucrose

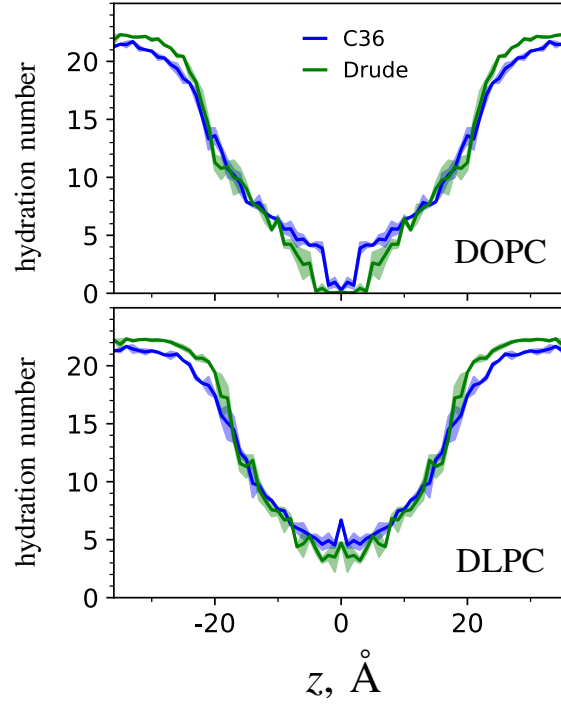


Figure B.11: Hydration number calculated from the trajectory of each window of the US simulations (including only one of the two $z = 0$ simulations), defined by the number of unique water oxygen within 3.5 Å of each sucrose hydroxyl oxygen. The errorbars are computed based on the expected symmetry of each membrane system. The distances were computed with MDAnalysis.^{235;236}

APPENDIX C

SUPPORTING INFORMATION FOR CHAPTER 4

C.1 Multilayer perceptron (MLP) loss function comparison

In Figure C.1, we provide an illustration of the effect of the choice of the loss function on the position-dependent diffusivity extracted by the MLP from the umbrella sampling molecular dynamics simulations. We compare the the mean-square error (MSE) given by the final optimization with each loss function,

$$\text{MSE}[C_{Fv}(t)] = \frac{1}{\mathcal{T}} \sum_{n=0}^{\mathcal{T}-1} (C_{Fv}(n\Delta t) - C_{Fv}^{MLP}(n\Delta t))^2 \quad (\text{C.1})$$

where $C_{Fv}(t)$ is the force-velocity time-correlation function obtained directly from the simulations and $C_{Fv}^{MLP}(t)$ is obtained from the convolution integral of the optimized memory function and the known velocity autocorrelation function. The simple loss function itself is given by eq C.1. The modified loss function is based on eq C.1, where the additional flat-bottomed harmonic terms based on the Woolf-Roux and Hummer diffusivity are added to it.

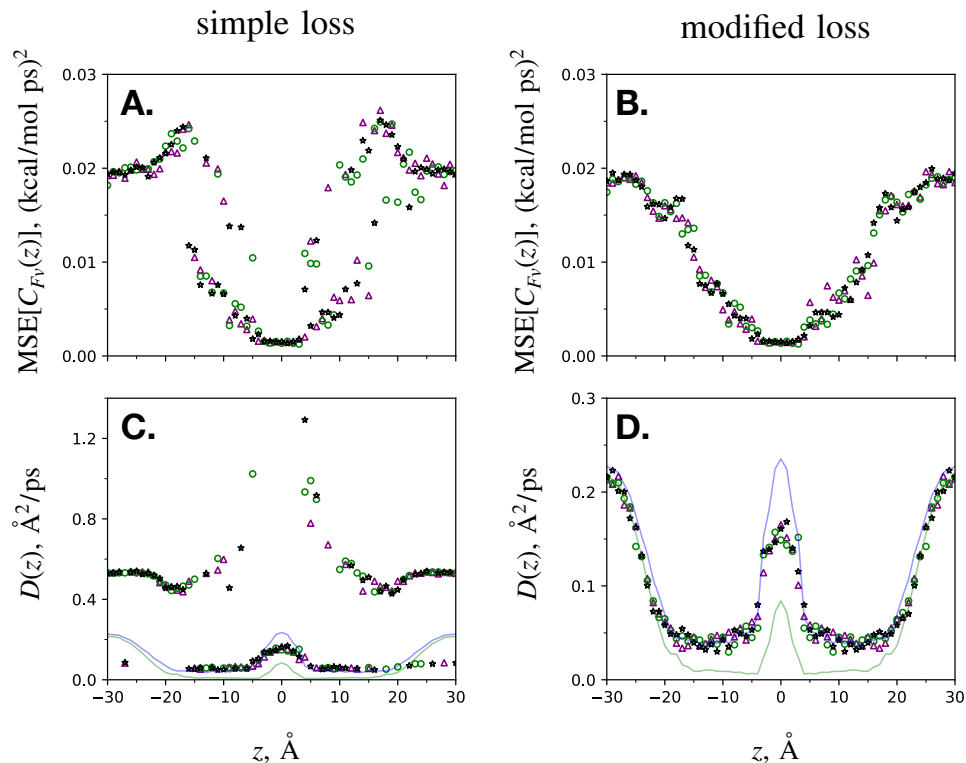


Figure C.1: Top row: The mean-square error in the force-velocity time-correlation function ($C_{Fv}(t)$) computed with eq C.1 for the MLP with the simple loss function of eq C.1 (A) and the modified loss function (B). Bottom row: The position-dependent diffusivity obtained by the MLP with the simple loss function (C) and the modified loss function (D). For reference, the Woolf-Roux (blue) and Hummer (green) diffusivity are displayed. For both loss functions, the optimization proceeds for 500 epochs using the Rprop method, and the initial parameters are randomized. The three distinct replicates for each window are displayed as purple triangles (1), green circles (2), and black stars (3).

C.2 Comparison of integration schemes for numerical generalized Langevin simulations

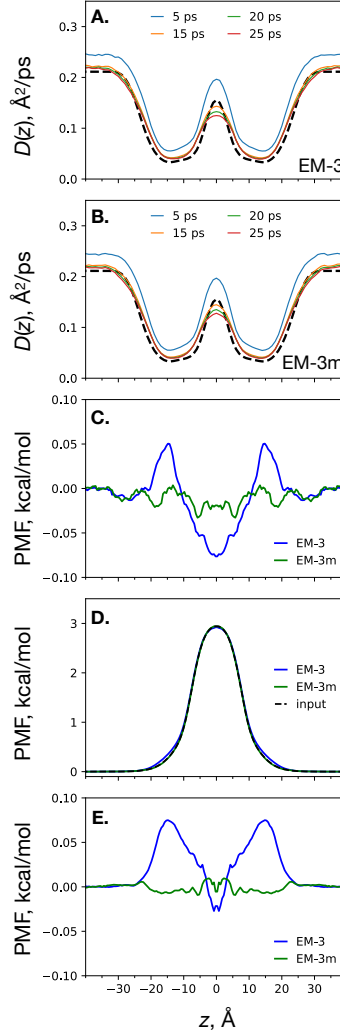


Figure C.2: Position-dependent diffusivity ($D(z)$) and PMF results of equilibrium simulations of the numerical GLE simulations with EM-3 and EM-3m integrator using the position-dependent memory function. **A.** EM-3 and **B.** EM-3m MSD $D(z)$ on flat free-energy surface for several lag times. The exact diffusivity of the underlying GLE (dashed black line). **C.** The PMF of the flat free-energy surface, or drift PMF, for EM-3 (blue line) and EM-3m (green line). **D.** The 3 kcal/mol barrier computed from the 1-D probability distribution with EM-3 (blue line) and EM-3m (green line), and the input barrier (black dashed line). **E.** The difference between the EM-3 (blue) and EM-3m (green-line) PMFs and the input PMF (drift PMFs). Each PMF is symmetrized about $z = 0$.

C.3 Multilayer perceptron (MLP) structure

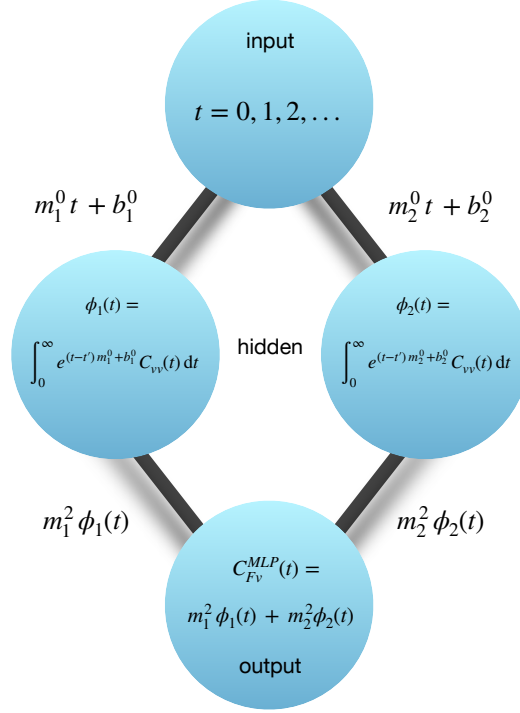


Figure C.3: Structure of the MLP developed by Russo et al.¹³⁴ for optimizing memory functions, with $N = 2$ neurons in the hidden layer. For the input layer, weights are represented by m_i^0 and the biases are represented by b_i^0 . In the hidden layer, the activator functions, $\phi_i(t)$ are the convolution integrals between the known velocity autocorrelation function, $C_{vv}(t)$, and $e^{m_i^0 t + b_i^0}$. The weights of the output layer are represented by m_i^2 . The output is the predicted C_{Fv}^{MLP} at the current iteration, which is given by $m_1^2 \phi_1(t) + m_2^2 \phi_2(t)$.

Based on the definitions of the weights and biases given in the structure of the MLP in Figure C.3, $1/\tau_i = -m_i^0$ and $K_i = m_i^2 \exp(b_i^0)$. The diffusivity can then be computed at a given iteration in terms of the weights and biases of the current state of the model,

$$D = -k_B T \left[\frac{m_1^2 e^{b_1^0}}{m_1^0} + \frac{m_2^2 e^{b_2^0}}{m_2^0} \right]^{-1} \quad (\text{C.2})$$

C.4 Time-correlation and memory functions (MD)

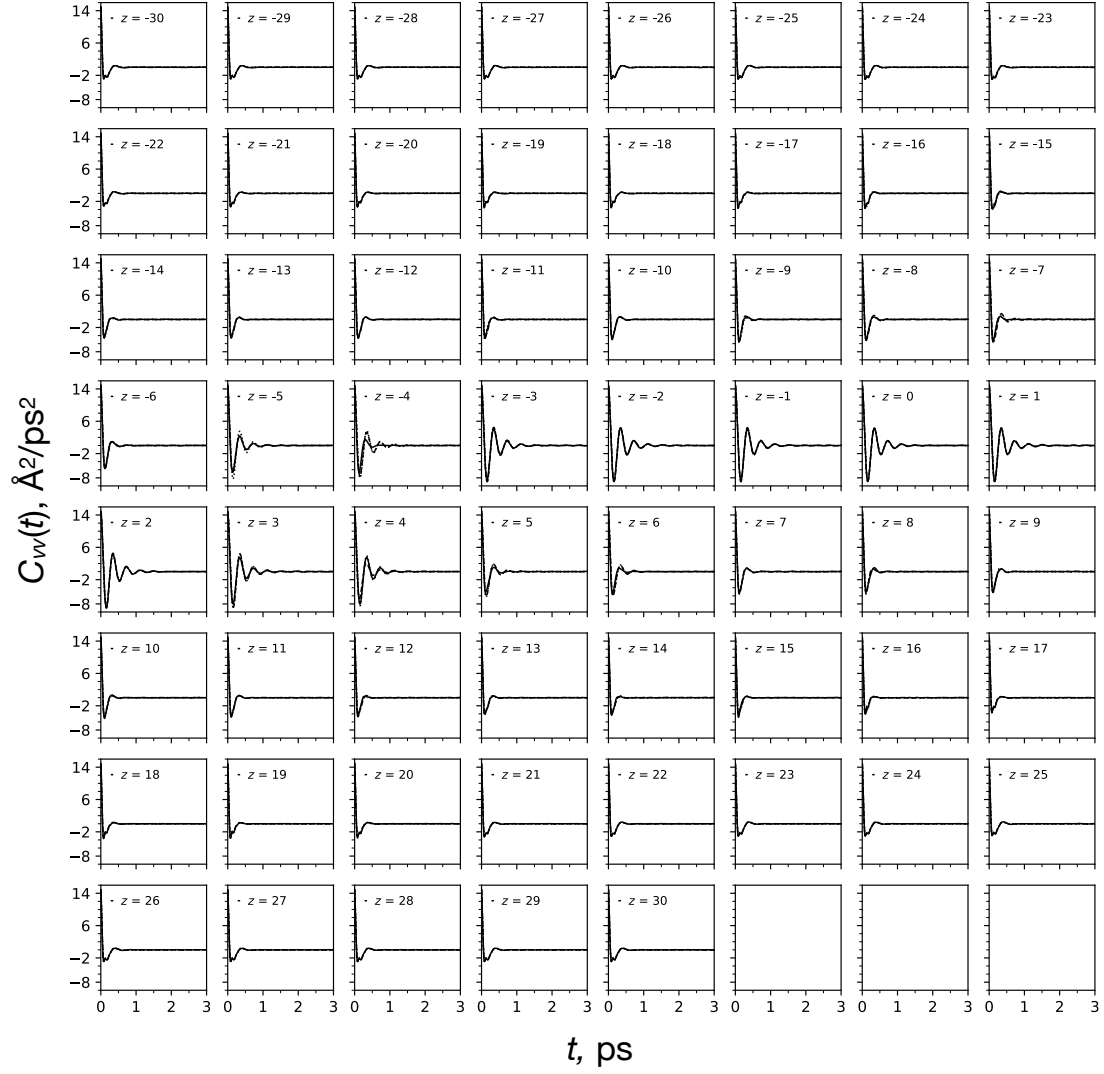


Figure C.4: Velocity autocorrelation function for each replicate and umbrella window from the MD simulations. Each replicate is shown in the same subplot with distinct line styles: solid (1), dashed (2), dotted (3).

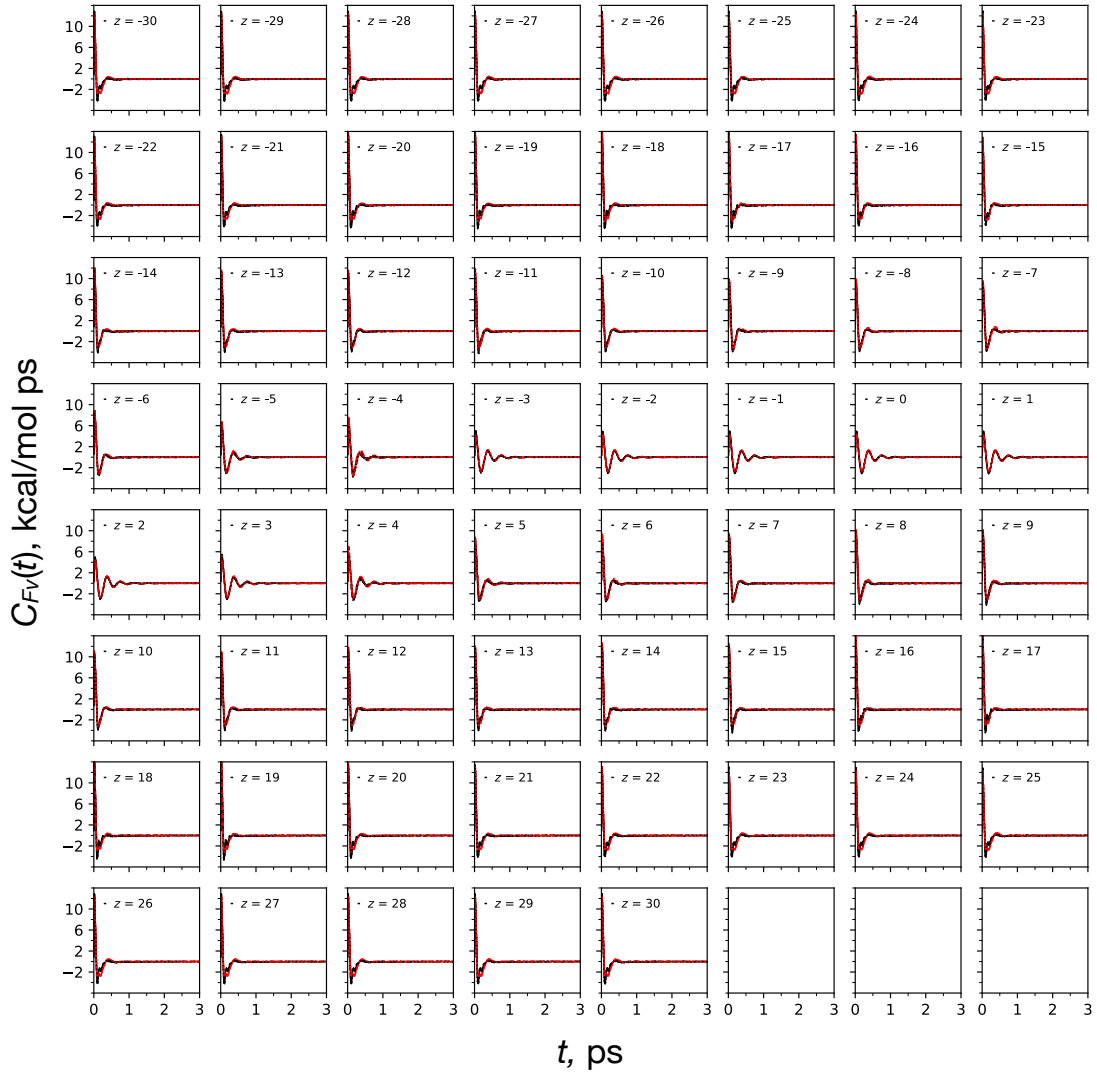


Figure C.5: Force-velocity ($C_{Fv}(t)$) time-correlation function for each replicate and umbrella window from the MD simulations (black). Also shown are the fitted time-correlation functions using each optimized memory function (red). Each replicate is shown in the same subplot with distinct line styles: solid (1), dashed (2), dotted (3).

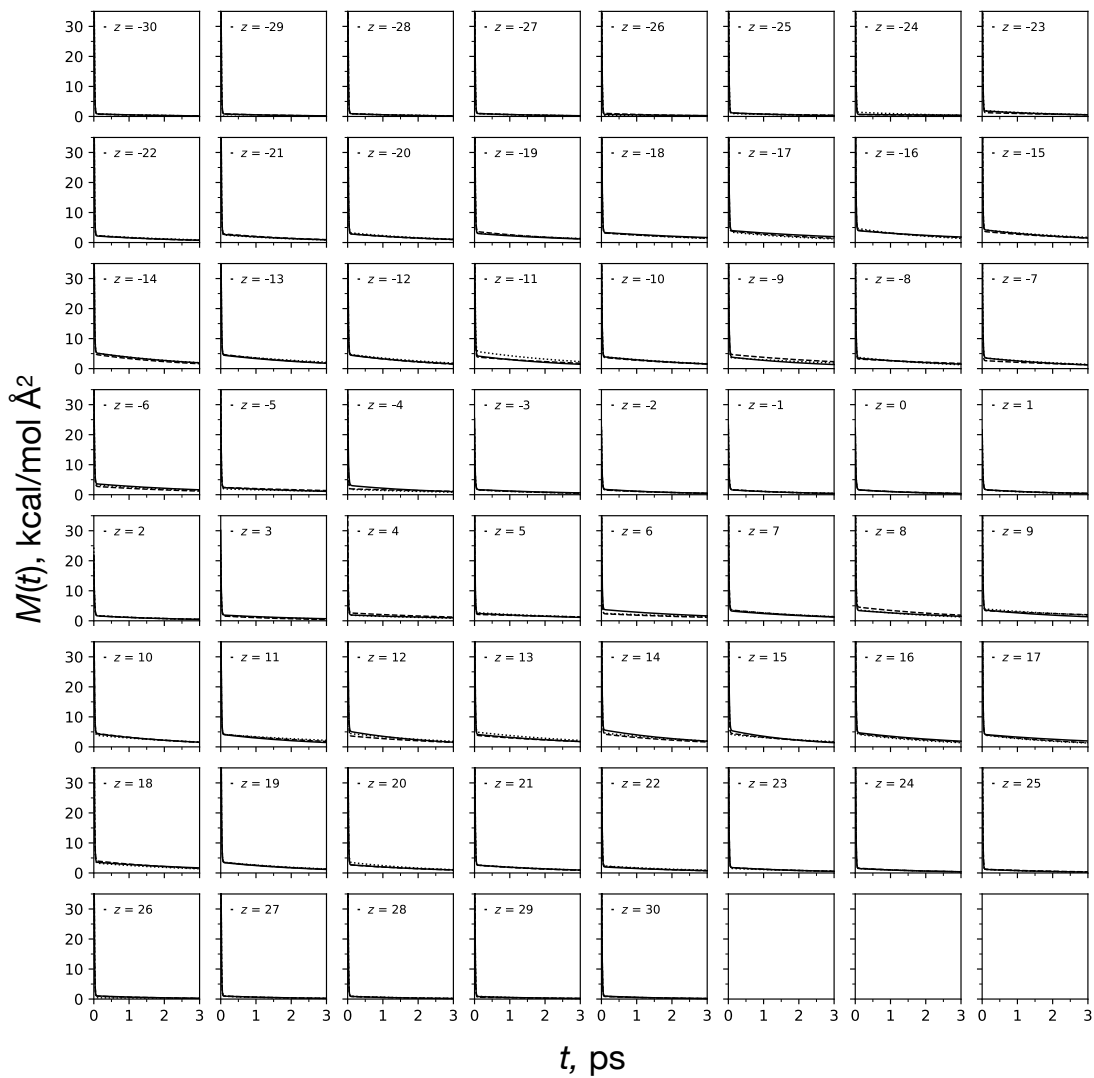


Figure C.6: Optimized memory functions for each replicate and umbrella window from the MD simulations. Each replicate is shown in the same subplot with distinct line styles: solid (1), dashed (2), dotted (3).

C.5 Woolf-Roux diffusivity extrapolation (MD)

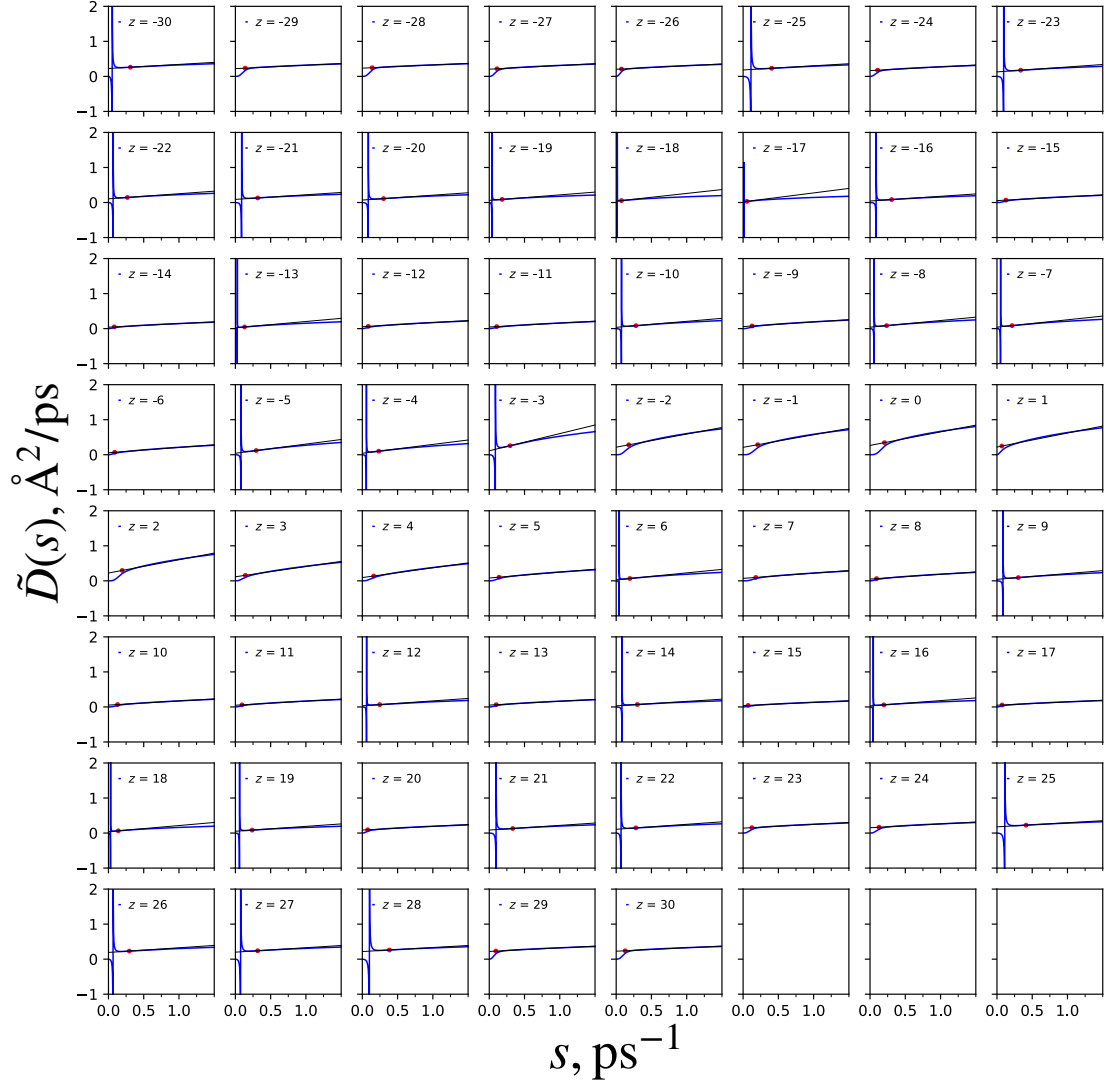


Figure C.7: Replicate 1: Laplace transform of the Woolf-Roux diffusivity from the MD simulations. The extrapolation to $s = 0$ for determining the final diffusivity value for each window is shown by the red dot (the location of fitting), and the black line.

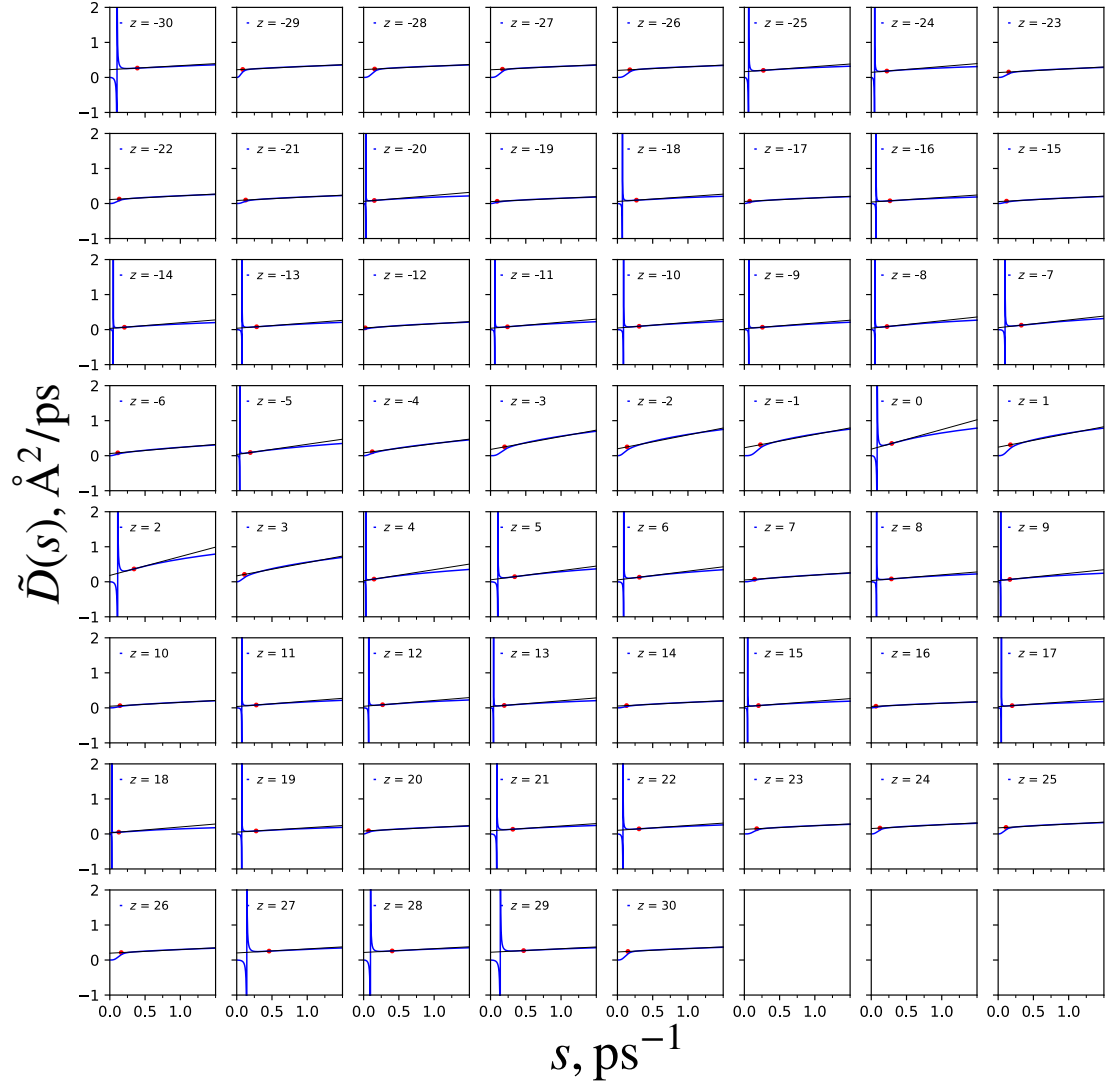


Figure C.8: Replicate 2: Laplace transform of the Woolf-Roux diffusivity from the MD simulations. The extrapolation to $s = 0$ for determining the final diffusivity value for each window is shown by the red dot (the location of fitting), and the black line.

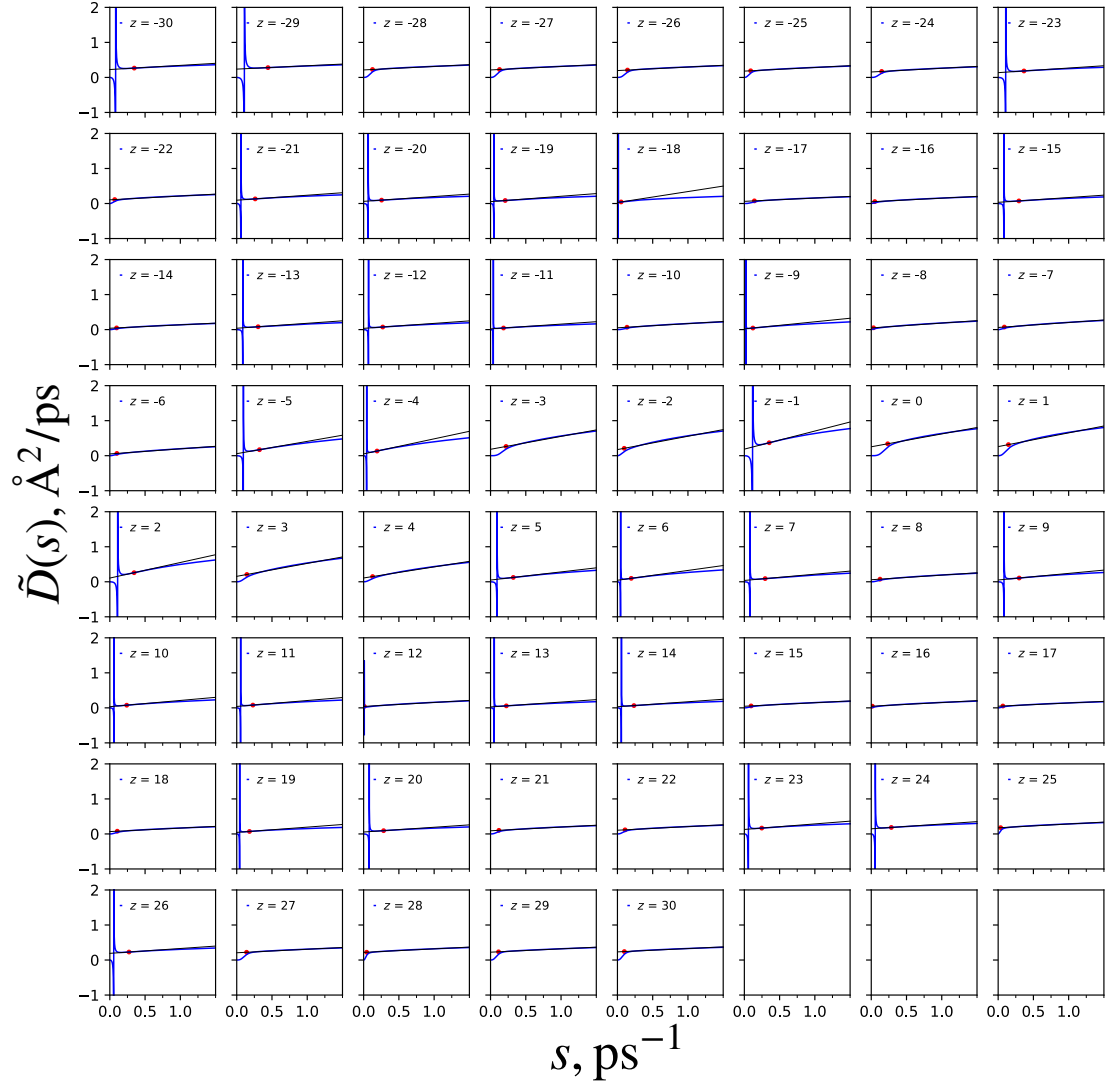


Figure C.9: Replicate 3: Laplace transform of the Woolf-Roux diffusivity from the MD simulations. The extrapolation to $s = 0$ for determining the final diffusivity value for each window is shown by the red dot (the location of fitting), and the black line.

C.6 Hummer diffusivity position autocorrelation function integrals (MD)

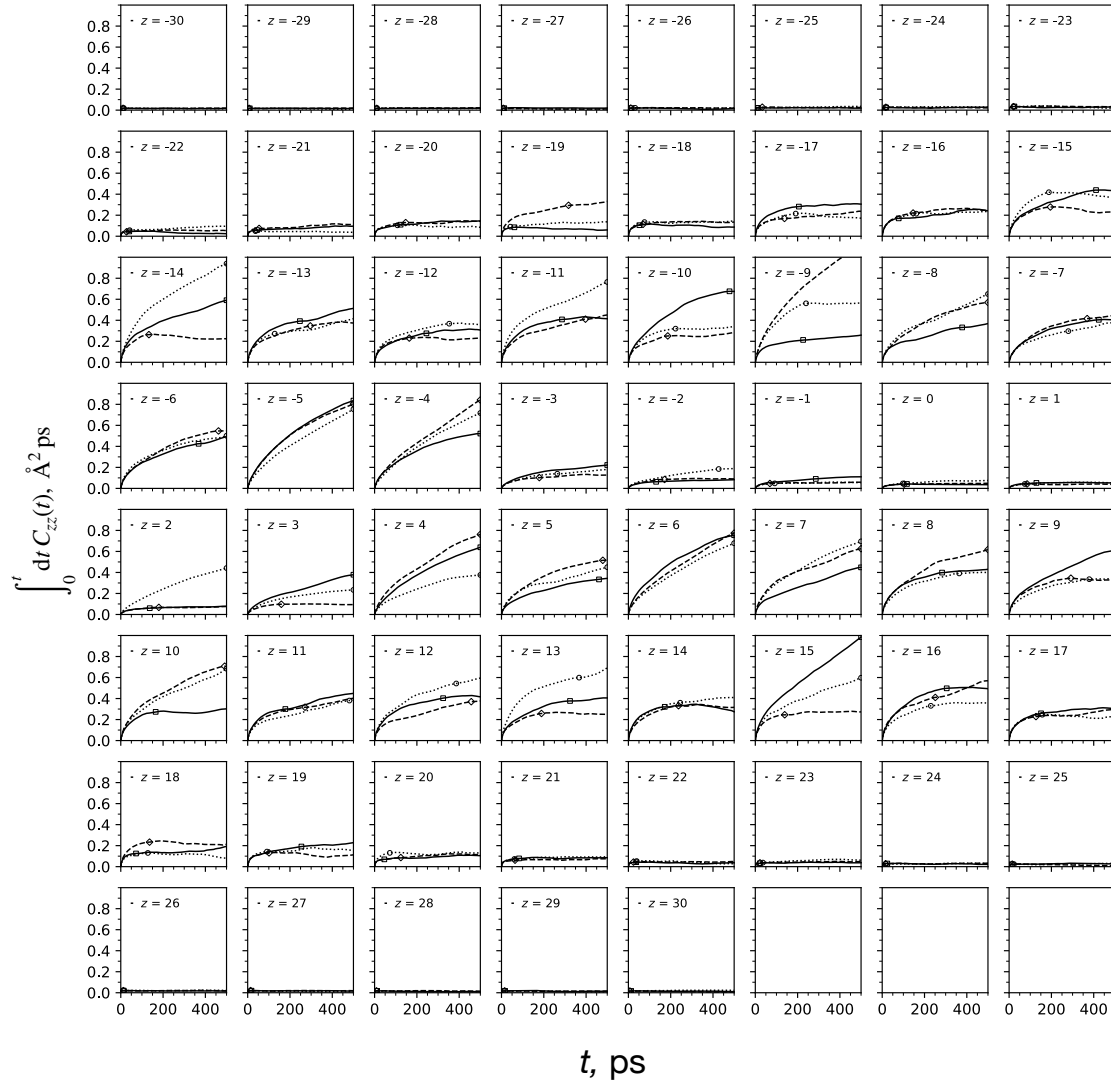


Figure C.10: The integral of the position-autocorrelation function for each window of the umbrella sampling MD simulations. Each subplot contains a line for each replicate: solid (1), dashed (2), dotted (3). The cutoffs of the upper limit of integration used in the final diffusivity ($t \rightarrow \infty$) are marked by the square (1), diamond (2), and circle (3) for each replicate.

C.7 Woolf-Roux diffusivity extrapolation (GLE)

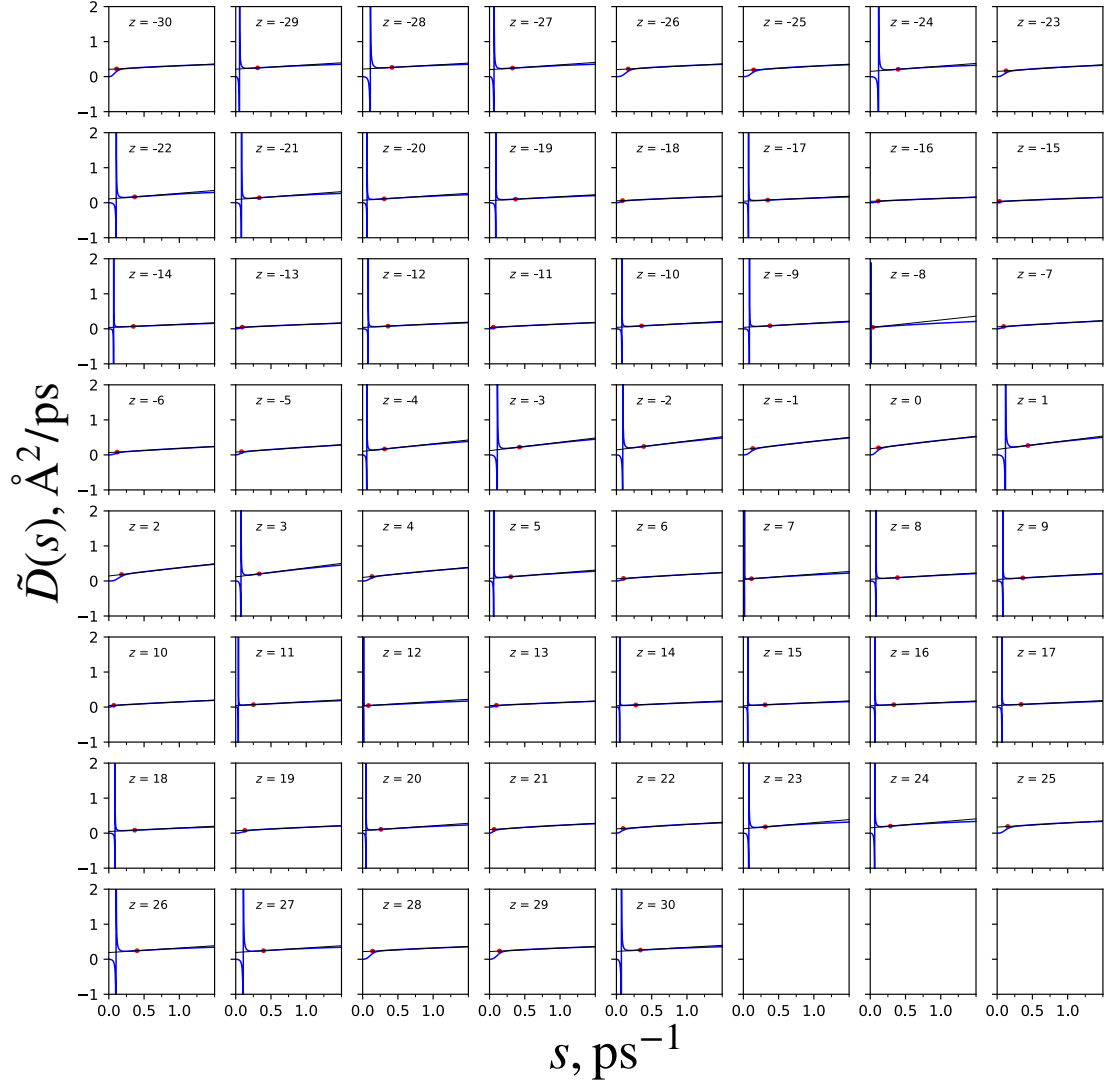


Figure C.11: Replicate 1: Laplace transform of the Woolf-Roux diffusivity from the GLE simulations. The extrapolation to $s = 0$ for determining the final diffusivity value for each window is shown by the red dot (the location of fitting), and the black line.

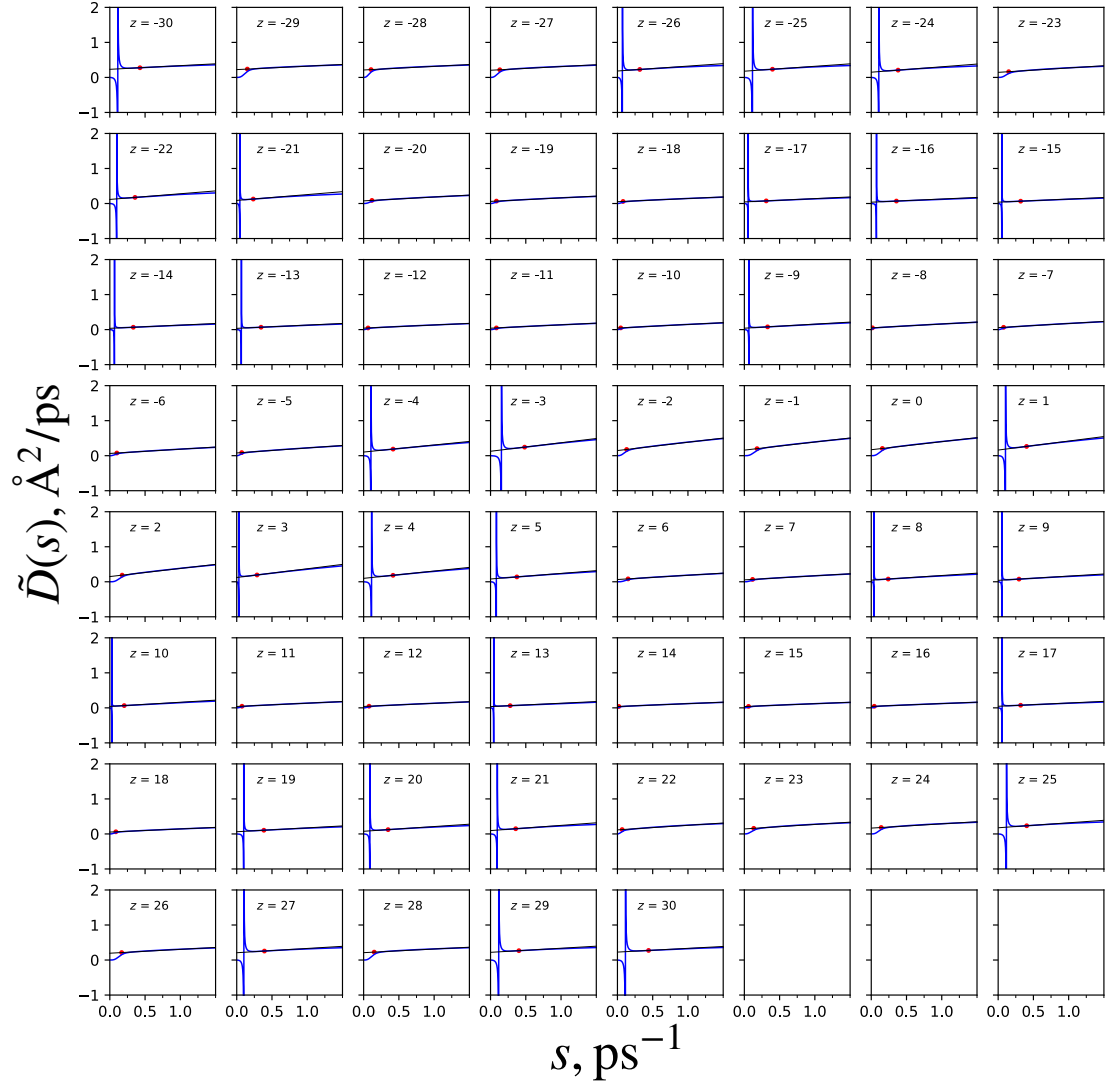


Figure C.12: Replicate 2: Laplace transform of the Woolf-Roux diffusivity from the GLE simulations. The extrapolation to $s = 0$ for determining the final diffusivity value for each window is shown by the red dot (the location of fitting), and the black line.

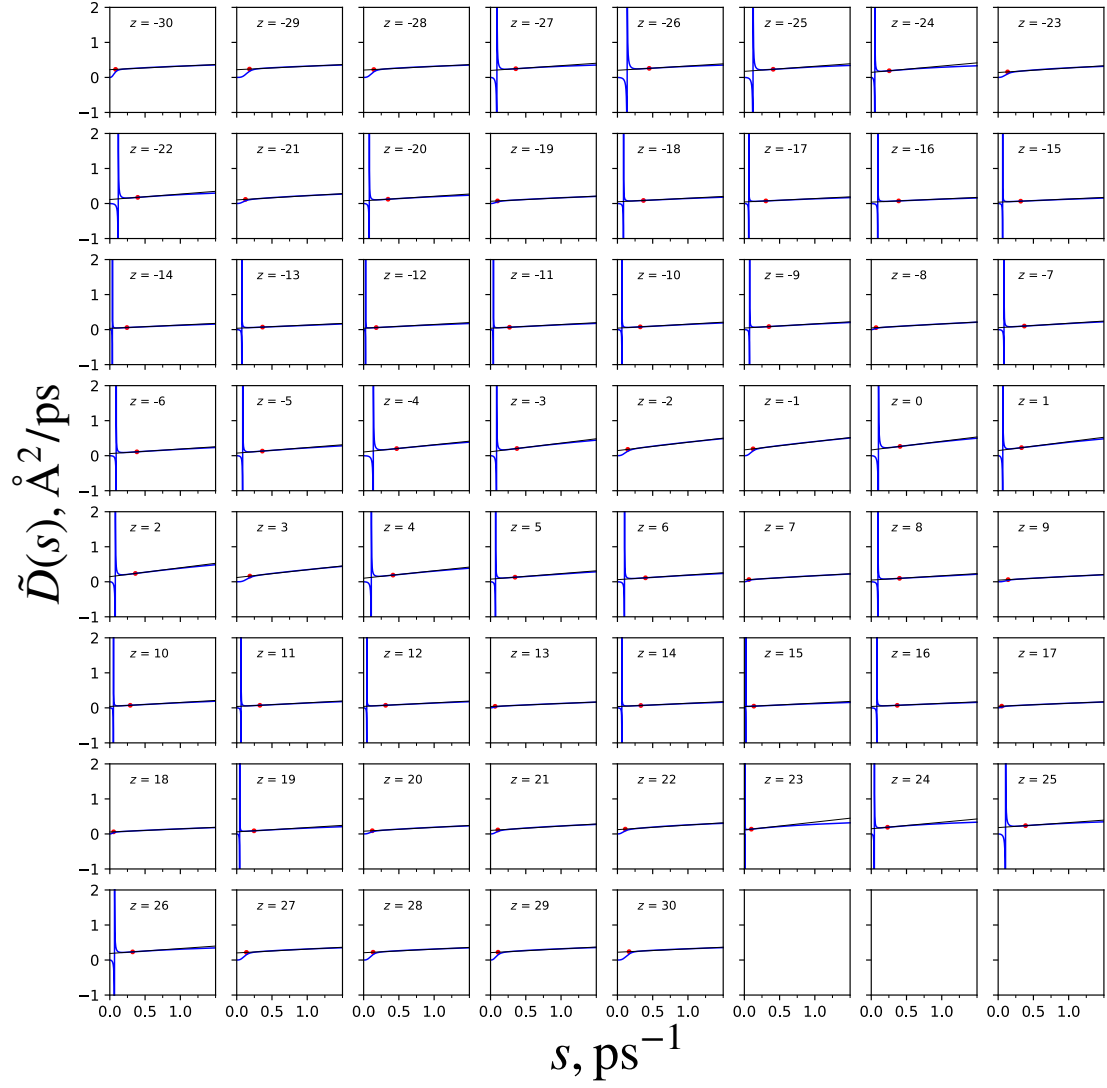


Figure C.13: Replicate 3: Laplace transform of the Woolf-Roux diffusivity from the GLE simulations. The extrapolation to $s = 0$ for determining the final diffusivity value for each window is shown by the red dot (the location of fitting), and the black line.

C.8 Hummer diffusivity position autocorrelation function integrals (GLE)

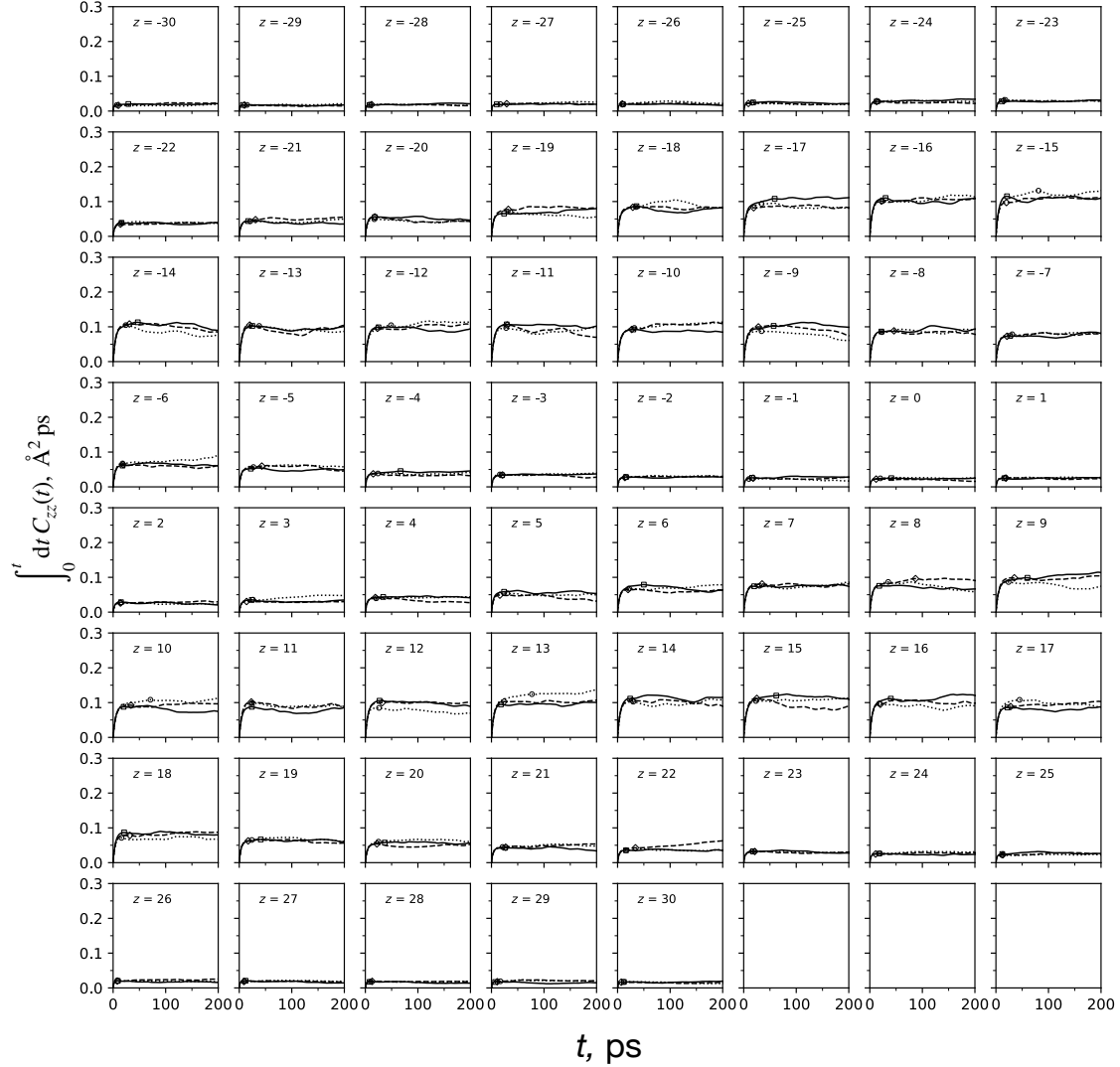


Figure C.14: The integral of the position-autocorrelation function for each window of the GLE umbrella sampling simulations. Each subplot contains a line for each replicate: solid (1), dashed (2), dotted (3). The cutoffs of the upper limit of integration used in the final diffusivity ($t \rightarrow \infty$) are marked by the square (1), diamond (2), circle (3) for each replicate.

C.9 Time-correlation functions (GLE)

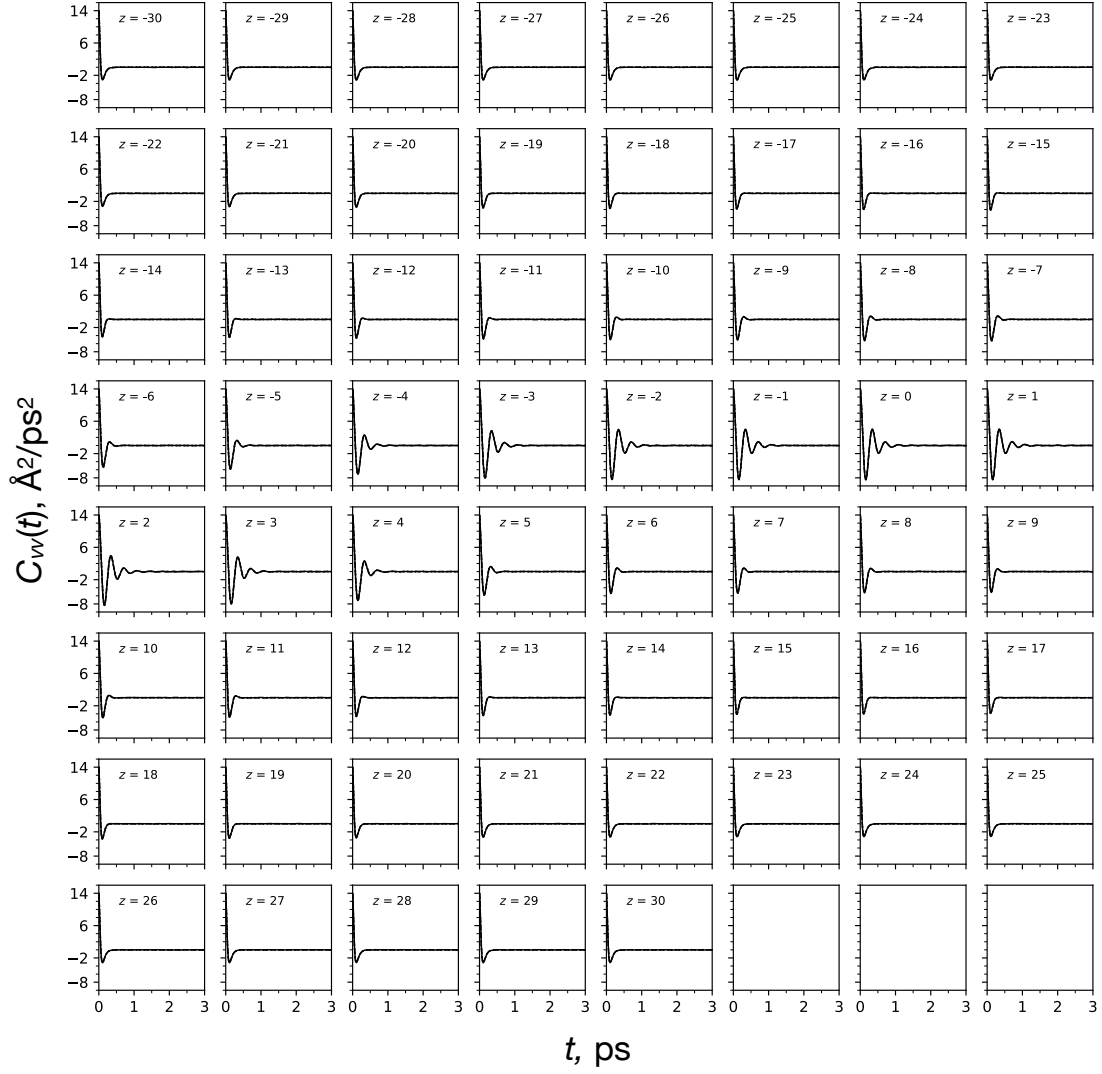


Figure C.15: Velocity autocorrelation function for each replicate and umbrella window of the GLE simulations. Each replicate is shown in the same subplot with distinct line styles: solid (1), dashed (2), dotted (3).

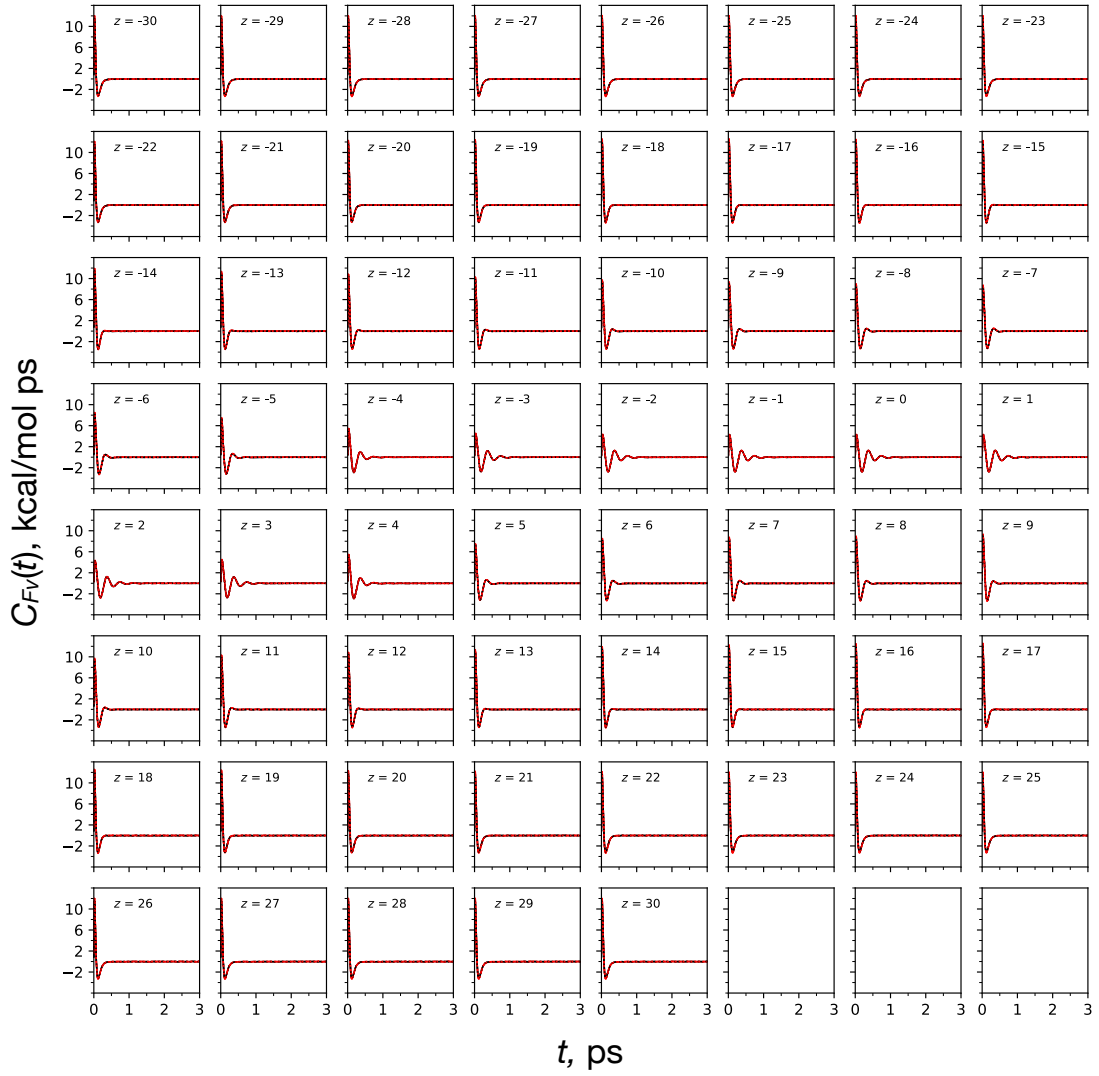


Figure C.16: Force-velocity ($C_{Fv}(t)$) time-correlation function for each replicate and umbrella window of the GLE simulations. Also shown are the fitted time-correlation functions using each optimized memory function (red) Each replicate is shown in the same subplot with distinct line styles: solid (1), dashed (2), dotted (3).

C.10 Grote-Hynes analysis of the MD $z = 0$ umbrella window

Within the context of Grote-Hynes theory, the permeability coefficient for a particle across a parabolic barrier is given by,¹³

$$P = \kappa \sqrt{\frac{k_B T}{2\pi m}} e^{-[W(z^\dagger) - W(-L/2)]/k_B T} \quad (\text{C.3})$$

where $W(z^\dagger)$ is the value of the PMF at the top of the barrier, $W(-L/2)$ is the PMF at the edge of the effective membrane, m is the particle's mass, and κ is the transmission coefficient.¹⁶ The value of κ is s/ω_0 , where $\omega_0 = \sqrt{-W''/m}$, W'' is the second derivative of the inverted parabolic PMF, and s is the real solution of,

$$\omega_0^2 - s^2 = \frac{s}{m} \tilde{M}(s) \quad (\text{C.4})$$

where $\tilde{M}(s)$ is the Laplace transform of the memory function. In the overdamped ($m \rightarrow 0$) and rapidly decaying memory ($\tilde{M}(s) \rightarrow k_B T/D(z^\dagger)$) limits, the solution of eq C.4 is, $s = -D(z^\dagger)W''/k_B T$. This solution results in $\kappa = (D(z^\dagger)/k_B T)\sqrt{-W''m}$, and a permeability exactly equal to the ISD model for a parabolic barrier and constant diffusivity.

We conducted Grote-Hynes analysis on the $z = 0$ MD umbrella window for the memory functions corresponding to Woolf-Roux, Hummer, and MLP in Laplace space. It was first necessary to make a harmonic approximation of the true PMF of water across the membrane. This was done using least-squares and yielded a W'' of $-0.087 \text{ kcal/mol } \text{\AA}^{-2}$ and a $W^\dagger$ of 5.93 kcal/mol . The harmonic PMF is shown in Figure C.17.

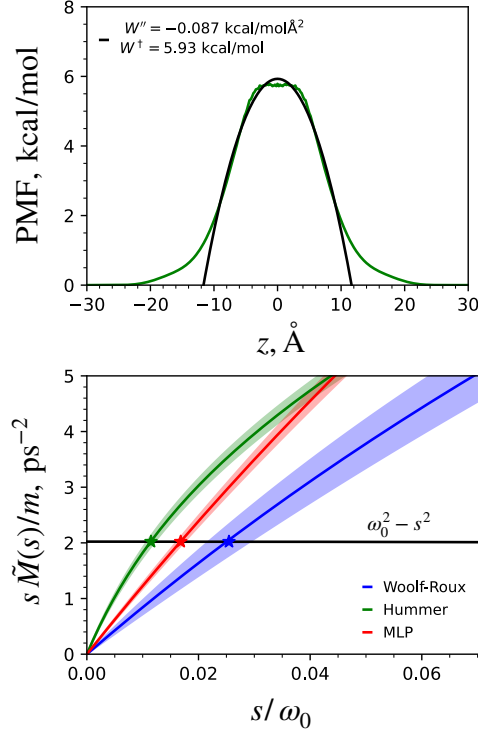


Figure C.17: Results of the Grote-Hynes analysis, using an inverted parabolic fit to the PMF from MD simulations of water permeation, and the memory functions extracted from the center $z = 0$ window of the umbrella sampling MD simulations. Top: The PMF of water across the DLPC membrane (green) and an inverted parabola fit by least-squares from $z = -10$ to $+10$ Å (black). The key parameters are the curvature, $W'' = -0.087$ kcal/mol Å² and the peak height, 5.93 kcal/mol. Bottom: The Grote-Hynes curve, $\omega^2 - s^2$, the left-hand side of eq C.4 (black), along with the right-hand side, $s\tilde{M}(s)/m$, for the Woolf-Roux (blue), Hummer (green), and MLP (red) memory functions. The stars indicate the mean s solution of the three replicates for each memory function. The uncertainty intervals are plus and minus the standard deviation of the three replicates.

We also evaluated eq C.3 in the high friction, overdamped limit to obtain the re-computed ISD (P_{OD}) permeability coefficients. These results, along with the values of $D(z^\ddagger)$ associated with each approach, are shown in Table C.1.

In each case, it was found that the increase in permeability obtained by applying Grote-Hynes theory is relatively small for Woolf-Roux ($\times 1.06$) and MLP ($\times 1.04$) and slightly larger for Hummer ($\times 1.34$). The marginal effect of the Grote-Hynes rate is evidence that memory effects are negligible for the permeation process. Additionally, it is revealed in Figure C.17 that $\omega_0^2 - s^2$ is nearly horizontal in the interval of s where the respective $s\tilde{M}(s)/m$ of Woolf-

Roux, Hummer, and MLP intersect. The lack of curvature from the quadratic function $(\omega_0^2 - s^2)$ indicates that the dynamics are overdamped on this timescale. The precise values of s where the intersections occur are given in Table C.1 for each model, falling between 0.01 and 0.04 ps⁻¹. Taken together, this analysis indicates that the permeation of water occurs in a predominately overdamped regime.

Table C.1: Grote-Hynes permeability from the Woolf-Roux, Hummer, and MLP Laplace space memory functions

	$P_{GH}, \times 10^{-3} \text{ cm/s}$	$P_{OD}, \times 10^{-3} \text{ cm/s}$	$D(z^\dagger), \text{ \AA}^2/\text{ps}$	$s, \text{ ps}^{-1}$
Woolf-Roux	20.0 ± 2.7	18.8 ± 2.8	0.235 ± 0.035	0.0362 ± 0.0049
Hummer	9.05 ± 0.56	6.73 ± 0.42	0.0841 ± 0.0052	0.0163 ± 0.0010
MLP	13.2 ± 0.56	12.67 ± 0.55	0.1583 ± 0.0069	0.0239 ± 0.0010

Notes: Uncertainty is the standard deviation of the three replicate simulations.

APPENDIX D

SUPPORTING INFORMATION FOR CHAPTER 6

D.1 Theory

We consider a membrane separating two solutions. The configuration of the system with a permeating molecule is determined by its position z and ionization state s . The equilibrium distribution in the membrane region, defined as $z \in [-L/2, L/2]$, is formally written as, $\pi_s(z)$. The probability density of the system at time t is expressed as $p_s(z, t)$. The forward propagation step $(z, s \rightarrow z', s')$ of the probability density from the time t to the time $t + \tau$ is,⁷⁰

$$p_s(z', t + \tau) = \sum_s \int dz \mathcal{P}_\tau(z', s' | z, s) p_s(z, t) \quad (\text{D.1})$$

where $\mathcal{P}_\tau(z', s' | z, s)$ represents the propagator for a transition from position z and state s to position z' and state s' over the lag time τ . It is assumed that the propagator obeys microscopic detailed balance, $\mathcal{P}_\tau(z, s | z', s') \pi_{s'}(z') = \mathcal{P}_\tau(z', s' | z, s) \pi_s(z)$. We define the left side of the membrane ($z \leq -L/2$) as the reactant state and the right side ($z \geq L/2$) as the product state. Under non-equilibrium steady-state conditions, the net unidirectional flux of permeating molecules from the left to the right side of the membrane is,⁶⁷

$$p_{s'}^{\text{ss}}(z') = \int dz \mathcal{P}_\tau(z', s' | z, s) p_s^{\text{ss}}(z) \quad (\text{D.2})$$

with $p_s^{\text{ss}}(z) = \pi_s(z)$ if $z \leq -L/2$ and $p_s^{\text{ss}}(z) = 0$ if $z \geq L/2$. It can be verified by direct substitution in eq D.2 that the steady-state density can be expressed as, $p_s^{\text{ss}}(z) = \pi_s(z) (1 -$

$q_s(z)$), where $q_s(z)$ is the committor defined as,

$$q_s(z) = \sum_{s'} \int dz' q_{s'}(z') \mathcal{P}_\tau(z', s' | z, s) \quad (\text{D.3})$$

with the constraints $q_s(z) = 0$ if $z \leq -L/2$, and $q_s(z) = 1$ if $z \geq L/2$. The committor is the probability that the system will first go to the product state (right side of the membrane) before going to the reactant state (left side of the membrane) when starting from position z and ionization state s .^{17;70;18} Substitution of $p^{\text{ss}}(z, s)$ in eq D.2 yields,

$$\begin{aligned} \pi_{s'}(z') (1 - q_{s'}(z')) &= \sum_s \int dz \mathcal{P}_\tau(z', s' | z, s) \pi_s(z) (1 - q_s(z)) \\ \pi_{s'}(z') (1 - q_{s'}(z')) &= \pi_{s'}(z') - \sum_s \int dz \mathcal{P}_\tau(z', s' | z, s) \pi_s(z) q_s(z) \\ q_{s'}(z') &= \sum_s \int dz q_s(z) \mathcal{P}_\tau(z, s | z', s') \end{aligned} \quad (\text{D.4})$$

where microscopic detailed balance was used. The steady-state flux from the left to the right side of the membrane can be expressed as the net transitions across a dividing surface at z^* separating the two sides,

$$\begin{aligned} J &= \frac{1}{\tau} \sum_{s', s} \int_{z \leq z^*} dz \int_{z' \geq z^*} dz' \left(\mathcal{P}_\tau(z', s' | z, s) p^{\text{ss}}(z, s) - \mathcal{P}_\tau(z, s | z', s') p^{\text{ss}}(z', s') \right) \\ &= \frac{1}{\tau} \sum_{s', s} \int_{z \leq z^*} dz \int_{z' \geq z^*} dz' \left(\mathcal{P}_\tau(z', s' | z, s) \pi_s(z) (1 - q_s(z)) - \mathcal{P}_\tau(z, s | z', s') \pi_{s'}(z') (1 - q_{s'}(z')) \right) \\ &= \frac{1}{\tau} \sum_{s', s} \int_{z \leq z^*} dz \int_{z' \geq z^*} dz' \left(q_{s'}(z') - q_s(z) \right) \mathcal{P}_\tau(z', s' | z, s) \pi_s(z) \end{aligned} \quad (\text{D.5})$$

where microscopic detailed balance was used. Because the steady-state flux does not depend on the position of the dividing surface z^* , it is convenient to choose a dividing surface corresponding to an isocommittor surface of $q_s(z)$ at some arbitrary value q^* . The flux from left to right can then be written as transitions from the point z with committor $q_s(z) < q^*$,

to the point z' with committor $q(z') > q^*$, and one can also integrate this expression over q^* from 0 to 1 because the steady-state flux $J_{AB}(q^*)$ does not actually depend on the specific value of q^* . This yields the quadratic expression

$$J = \frac{1}{2\tau} \sum_{s',s} \int dz \int dz' \left(q_{s'}(z') - q_s(z) \right)^2 \mathcal{P}_\tau(z', s' | z, s) \pi_s(z) \quad (\text{D.6})$$

(the factor of 2 is needed when the restriction $q_{s'}(z') > q_s(z)$ is removed).

D.2 Enhanced sampling convergence

Figure D.1 contains the unsymmetrized gradients of the center of mass of each molecule along

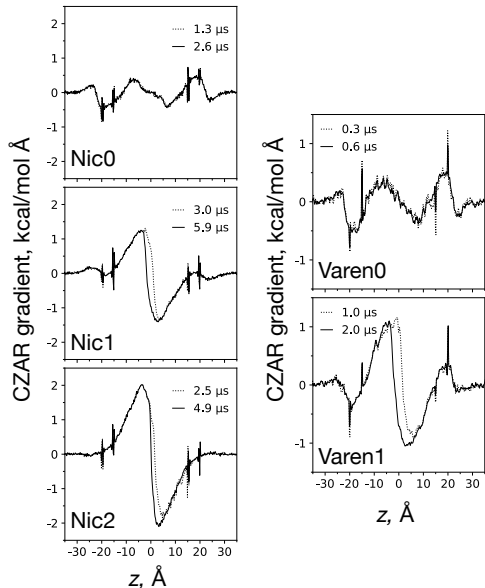


Figure D.1: CZAR gradient²⁰³ output from the eABF algorithm in colvars in NAMD 2.14^{194;195} for each ionization state of nicotine and varenicline. The gradients are shown before antisymmetrization. The solid lines are the final gradients, and the dotted lines are the gradients after half of the total simulation time.

the membrane normal which were computed by WTM-eABF enhanced sampling, and output by NAMD 2.14 colvars with the CZAR method.^{194;195;203} The figure shows the convergence of each simulation from half of the total time to the total time. Similarly, Figure D.2 shows

the sampling histogram of each molecule, or the number of times the molecule visited each

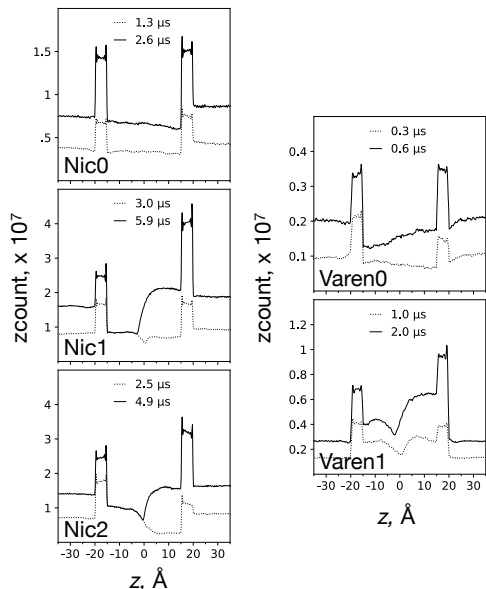


Figure D.2: Histogram of eABF sampling used for computing the CZAR gradients,²⁰³ output as zcount from colvars in NAMD 2.14,^{194;195} shown for each ionization state of nicotine and varenicline. The solid lines are the final sampling histograms, and the dotted lines are the sampling from half of the total simulation time.

bin along the colvar, z , throughout the enhanced sampling simulation. Figure D.3 shows the unsymmetrized PMFs which were output from NAMD 2.14 colvars with the CZAR method.^{194;195;203}

D.3 Lag time dependence of diffusivity

D.3.1 MSD diffusivity

The diffusivity computed by local mean-square derivations (MSD) over the membrane normal for a range of lag times is shown for each molecule in Figure D.4. The lag time dependence of the MSD diffusivity in the membrane region is evidence of subdiffusive behavior of the molecule.⁴⁹ In such situations, it can be reasonably assumed that longer lag times will

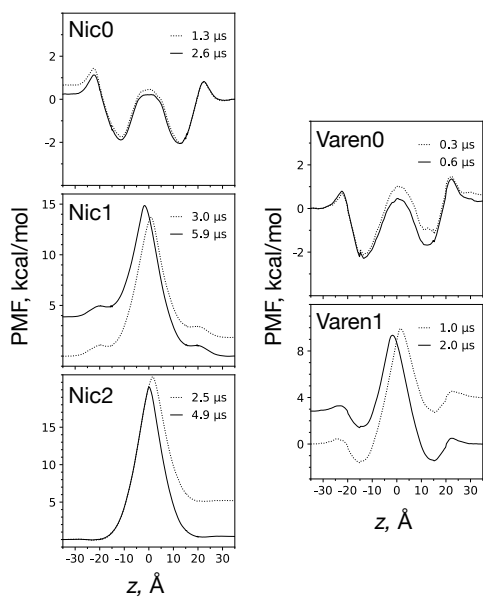


Figure D.3: Unsymmetrized PMFs, as output from the NAMD 2.14 colvars^{194;195} simulation for each ionization state of nicotine and varenicline. The convergence of the PMFs over time can be seen in the progression from half of the total simulation time (dotted lines) to the end of the simulation (solid lines).

provide more accurate local diffusivity estimates, however, as lag time increases, position dependence is gradually lost.

D.3.2 Time-fractional Smoluchowski model

To assess the validity of the choice of 20 ps for offering a reasonable balance of position dependence, we used the time-fractional Smoluchowski diffusion equation as implemented in the DiffusionFusion software [<https://github.com/jeffcomer/DiffusionFusion#readme>], written by Jeffery Comer and available on GitHub^{49;237}. To compute the time-fractional position-dependent diffusivity with DiffusionFusion, we used the Crank-Nicholson solver. The input trajectory consisted of the same converged eABF simulations that were used for the MSD diffusivity calculation. The input forces were therefore neglected, or assumed to be zero. The alpha value, or the position-dependent fractional exponent of the the time dimension of the diffusivity, was optimized along with the position-dependent diffusivity. The values

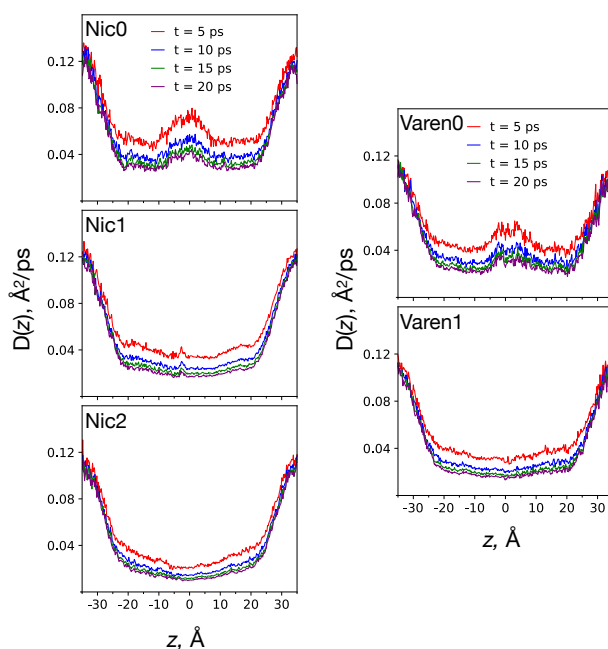


Figure D.4: Mean-square deviation (MSD) diffusivity for all ionization states of nicotine and varenicline before symmetrization. Each color indicates a different lag time used for computing the local displacements. The lag time dependence is evident for each molecule near the center of the membrane.

were computed in $0.5 \text{ \AA}$ bins. In order to best minimize lag time dependence, two lag times were considered in one calculation. To compute the diffusivity for a lag time of 5 ps, the trajectory was input with a lag time of both 5 and 10 ps. To compute the diffusivity with a lag time of 10 ps, the trajectory with both a lag time of 10 and 20 ps was used.

The resulting time-fractional diffusivity profile for lag times of 5 and 10 ps are shown for each ionization state of nicotine on the left side of Figure D.5. Also shown is the MSD diffusivity for a lag time of 20 ps (used in main text for permeability calculations). On the right side of Figure D.5 are the alpha values associated with the time-fractional diffusivity for each lag time. Alpha values close to 1 can be roughly interpreted as evidence that there is little subdiffusive behavior. We observed that for the neutral molecule, the alpha value dips below unity near the center of the membrane. This deviation is diminished for the singly protonated molecule, and essentially negligible for the doubly protonated molecule. The MSD diffusivity for 20 ps, therefore, is in good agreement with the time-fractional diffusivity

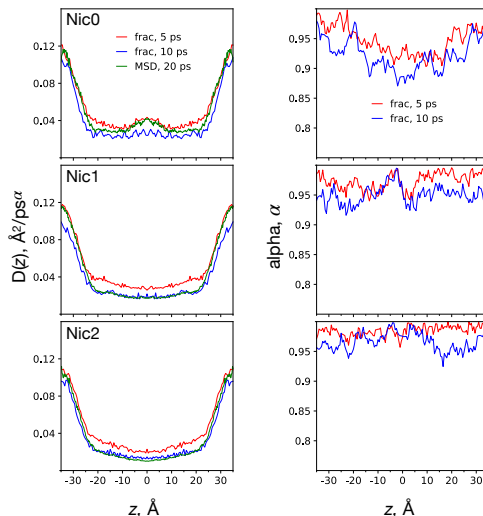


Figure D.5: Time-fractional position dependent diffusivity⁴⁹ (left) for lag times of 5 (red) and 10 ps (blue), along with the MSD diffusivity for a lag time of 20 ps (green) for the three ionization states of nicotine. The fractional exponent of the time dimension of the time-fractional diffusivity, α (right), for a lag time of 5 ps (red) and 10 ps (blue). For singly and doubly protonated nicotine, the value of α remains near unity over the entire z coordinate, indicating that there is a smaller effect of subdiffusion for these molecules.

for the two protonated molecules, while there is some difference between them near the center of the membrane for the neutral molecule.

D.3.3 Investigation of ionization state dependence of the diffusivity profile

Because the three ionization states of nicotine only differ from each other by a single hydrogen atom, it is worth considering why the diffusivity for each differs near the center of the membrane, and why the protonated molecules have less subdiffusive behavior. One potential explanation is the increased water density inside the membrane in the converged eABF trajectories of the protonated molecules. In Figure D.6A,

the PMF of water from the converged eABF simulation of each ionization state of nicotine is shown. The PMFs were computed based on the probability distribution of finding water molecules in bins along the membrane normal over the entire trajectory. For the neutral molecule, the water barrier is close to 7 kcal/mol, which is near the previously established barrier for POPC membranes.¹ For the singly and doubly protonated molecules, the barrier

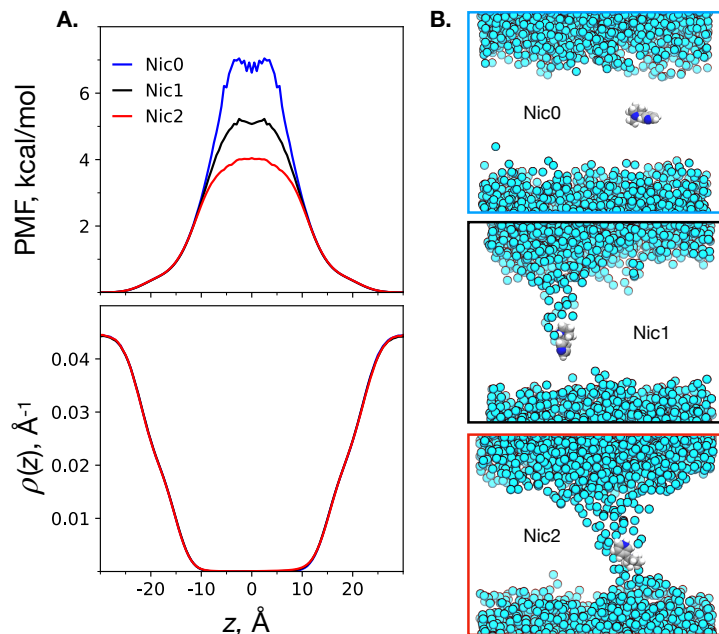


Figure D.6: **A.** PMFs of water (top) computed from the marginal probability distribution of water (bottom) in bins along the membrane normal from the converged eABF simulations for each of the three ionization states of nicotine. The lower barrier for the singly and doubly protonated forms indicates that there is a higher water density in the biased simulations of these molecules compared to the neutral molecule. **B.** Snapshots from the converged eABF simulations for each ionization state of nicotine, illustrating the extent to which water enters the membrane in each instance. The water oxygen atoms are represented by cyan circles. The nicotine molecules are shown in the VDW representation. The snapshots were made with VMD.¹⁹⁷

is substantially lowered, which is evidence that the charged molecules enter the membrane while accompanied by water molecules. Further evidence of this phenomenon is shown by the snapshots of nicotine in the membrane in the converged eABF simulations in Figure D.6B. We also computed hydration numbers for each ionization state of nicotine along the z -axis to quantify the observation that the charged forms enter the membrane with more water molecules, show in Figure D.7. The hydration number was computed by the total unique number of water oxygen atoms within 3.5 Å of each nicotine nitrogen atom, using the same cutoff as Yue et al.²⁷ We found that the mean hydration number for deprotonated and singly protonated nicotine is roughly 3.5 in bulk solution, while for doubly protonated nicotine it is around 4. Inside the membrane, there is a deviation between all three states, with the deprotonated molecule reaching a hydration number of essentially 0, singly protonated around

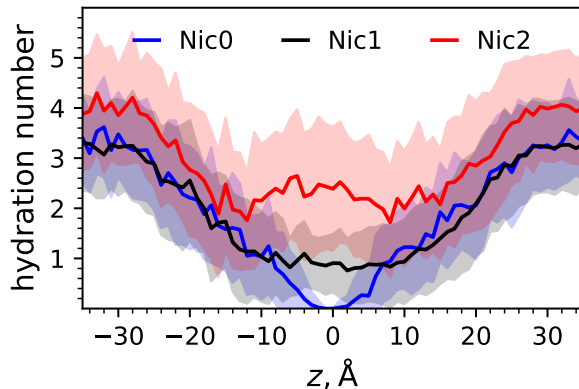


Figure D.7: Hydration numbers for each ionization state of nicotine along the z -axis. The hydration number was computed by the total number of unique water oxygen atoms within $3.5 \text{ \AA}$ of each of the two nitrogen atoms of nicotine. The mean hydration number was computed in $1 \text{ \AA}$ bins over each frame of the converged eABF trajectories (also used for diffusivity calculations). The error bars are the standard deviation of the hydration numbers computed for each frame. The nitrogen–oxygen distances were computed with MDAnalysis.^{235;236}

1, and doubly protonated about 2.5. These observations are in line with the behavior of both the MSD and fractional diffusivity profiles. The MSD diffusivity in the membrane is smallest for the bulky doubly protonated molecule, and largest for the dehydrated deprotonated molecule. Further, the fractional diffusivity shows the greatest deviation from the MSD diffusivity for the deprotonated state, which is in line with the concept that a larger hydration number decreases the effects of subdiffusion by increasing the effective size of the molecule.

D.4 Boltzmann-weighted average diffusivity

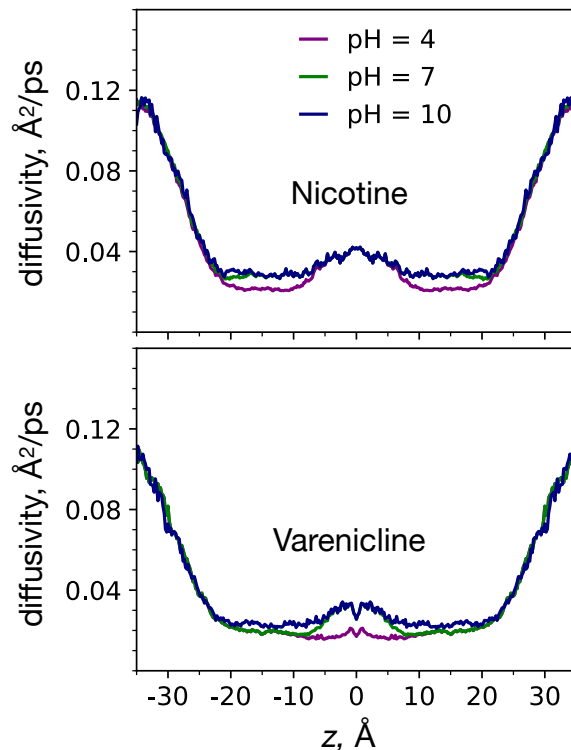


Figure D.8: Boltzmann-weighted average diffusivity profiles over a range of pH values for Nicotine (top) and varenicline (bottom). The profiles were computed at each pH by eq 17 in the main text and used for calculating the corresponding BWAAP permeability.

D.5 Derivation of empirical relationship between protonated and neutral state contributions to pH-dependent permeability

We aim to demonstrate the relative contribution of each ionization state to the effective pH-dependent permeability over a two-dimensional surface with pK_a and the difference in barrier heights as free variables, expressed as $\log \frac{f_1 P_1}{f_0 P_0}$. We begin with analytical PMFs for a generic neutral or protonated molecule,

$$W_s(z) = W_s^\dagger e^{-(2z/d)^2} \quad (\text{D.7})$$

where $W_s^\dagger$ is the barrier height of state s , and d is approximately the width of the membrane. We can also define a generic diffusivity profile, which gradually dips to a broad minimum

inside the membrane,

$$D(z) = -(D_{aq} - D_{memb})e^{-(z/d)^6} + D_{aq} \quad (\text{D.8})$$

where D_{aq} and D_{memb} are the aqueous and membrane diffusivity, respectively. The PMFs and diffusivity are shown in Figure D.9A. and B.

The value of the empirical function across $\text{p}K_a$ and barrier heights will approximate $\log \frac{f_1 P_1}{f_0 P_0}$. We rewrite the logarithm in terms of $\text{p}K_a$, pH , PMF, and diffusivity,

$$\log \frac{f_1 P_1}{f_0 P_0} = \log \left[\frac{\int_{-d/2}^{d/2} dz e^{W_0(z)/k_B T} / D(z)}{\int_{-d/2}^{d/2} dz e^{W_1(z)/k_B T} / D(z)} \right] + \text{p}K_a - \text{pH} \quad (\text{D.9})$$

We observe that the logarithm of the permeability scales approximately linearly for an intermediate range of barrier heights, $W_s^\dagger$. We can then express the empirical form of the relative contribution of each ionization state,

$$\log \frac{f_1 P_1}{f_0 P_0} = C(W_0^\dagger - W_1^\dagger) + \text{p}K_a - \text{pH} \quad (\text{D.10})$$

where C is an empirical constant. The constant C was obtained by a linear fit to the permeability calculated over a range of barrier heights from 0 to 10 kcal/mol, as shown in Figure D.9C. Given a C value of $0.64 \text{ (kcal/mol)}^{-1}$, or $0.39 / k_B T$, we validated eq D.10 for a range of barrier heights and $\text{p}K_a$ values, with a fixed pH of 7, against the explicit evaluation of eq D.9. The empirical two-dimensional surface of relative contributions to the effective permeability is shown in Figure D.9D.

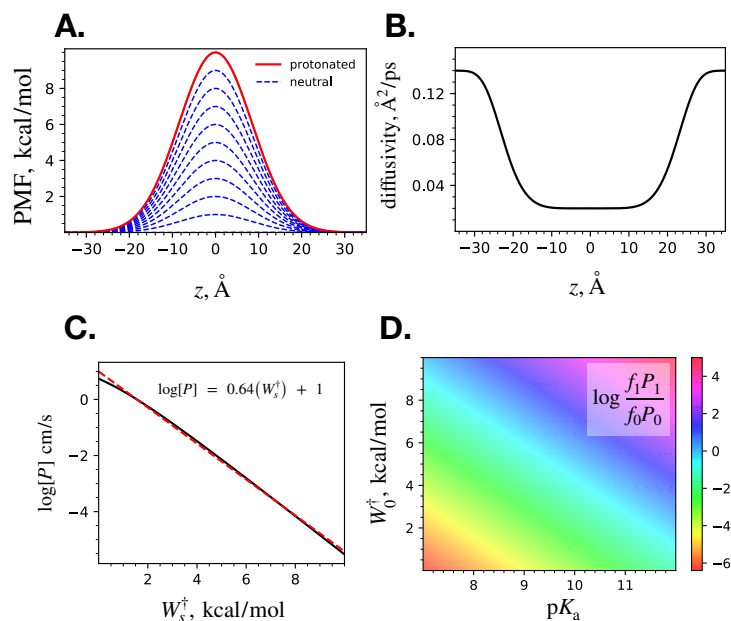


Figure D.9: **A.** PMFs generated with eq D.7 for several barrier heights. The red line indicates the fixed protonated form PMF, which was used for computing **D.** as the neutral barrier increases. **B.** Diffusivity profile generated with eq D.8 used for computing the ISD permeability of either state. **C.** Logarithm of permeability computed over a range of barrier heights (solid black), with linear fit (dotted red). The slope of the line is $0.64 \text{ (kcal/mol)}^{-1}$ and the y -intercept is 1 log[cm/s] . **D.** Evaluation of eq D.10 over a range of pK_a and neutral barrier heights, where the protonated barrier height is fixed at 10 kcal/mol. The empirical constant C was set to $0.64 \text{ (kcal/mol)}^{-1}$, and the pH was set to 7.

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