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An Atomic and Molecular View of the Depth Dependence of the Free Energies of Solute Transfer from Water into Lipid Bilayers

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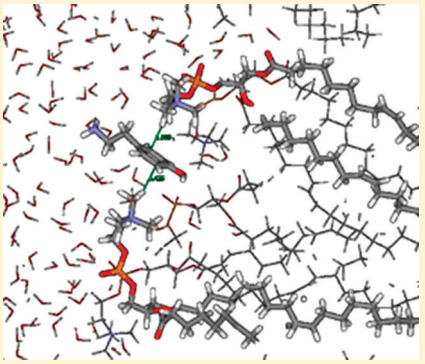
ABSTRACT: Molecular interactions and orientations responsible for differences in 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) bilayer partitioning of three structurally related drug-like molecules (4-ethylphenol, phenethylamine, and tyramine) were investigated. This work is based on previously reported molecular dynamics (MD) simulations that determined their transverse free energy profiles across the bilayer. Previously, the location where the transfer free energy of the three solutes is highest, which defines the barrier domain for permeability, was found to be the bilayer center, while the interfacial region was found to be the preferred binding region. Contributions of the amino (NH2) and hydroxyl (OH) functional groups to the transfer free energies from water to the interfacial region were found to be very small both experimentally and by MD simulation, suggesting that the interfacial binding of these solutes is hydrophobically driven and occurs with minimal loss of hydrogen-bonding interactions of the polar functional groups which can occur with either water or phospholipid head groups. Therefore, interfacial binding is relatively insensitive to the number or type of polar functional groups on the solute. In contrast, the relative solute free energy in the barrier domain is highly sensitive to the number of polar functional groups on the molecule. The number and types of hydrogen bonds formed between the three solutes and polar phospholipid atoms or with water molecules were determined as a function of solute position in the bilayer. Minima were observed in the number of hydrogen bonds formed by each solute at the center of the bilayer, coinciding with a decrease in the number of water molecules in DOPC as a function of distance away from the interfacial region. In all regions, hydrogen bonds with water molecules account for the majority of hydrogen-bonding interactions observed for each solute. Significant orientational preferences for the solutes are evident in certain regions of the bilayer (e.g., within the ordered chain region and near the interfacial region 20−25 Å from the bilayer center). The preferred orientations are those that preserve favorable molecular interactions for each solute, which vary with the solute structure.



KEYWORDS: *partitioning, permeation, membrane, bilayer, drugs, simulation, molecular dynamics, transport*

INTRODUCTION

Drug distribution in various tissues and the effective drug concentration at the intended site of action are governed in part by molecular transport across and partitioning into lipid bilayers that constitute the membranes of cells and intracellular organelles. Also, the in vivo performance of lipid bilayer-containing drug delivery systems such as liposomes depends directly on their ability to entrap and retain drug molecules for extended periods of time before their release. Consequently, understanding the energetics of transport across and retention of drug molecules in lipid bilayers is of significant value in developing a molecular level understanding of both drug delivery kinetics and distribution.

According to bulk solubility−diffusion theory, which assumes that a lipid bilayer resembles a thin homogeneous slab of bulk solvent,[1,2] the passive lipid bilayer permeability of a molecule is proportional to its membrane/water partition coefficient and membrane diffusion coefficient. However, numerous experimental studies as well as molecular dynamics (MD) simulations have drawn attention to the existence of distinct regions within bilayers with differing chemical properties[3] indicative of significant bilayer heterogeneity. One manifestation of bilayer heterogeneity is that the relative *permeabilities* of a series of solutes correlate with their hydrocarbon/water partition coefficients, indicating that the barrier domain within bilayers is the hydrocarbon-like interior,[4] while membrane *binding affinities* are much less sensitive to solute structure and more closely correlated to partition coefficients between water and relatively polar organic solvents.[5]

Bilayer heterogeneity leads to polarity gradients and free energy profiles that are position-dependent, varying as a function of depth within the bilayer, as reported in several

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recent MD simulations.[6−11] *Inhomogeneous* solubility−diffusion models developed more recently to account for local heterogeneity within bilayers have been used to determine permeability coefficients of relatively small molecules (e.g., water, methanol, ethane, benzene) from their free energy profiles and position-dependent diffusion coefficients.[3,10,12,13] While such information is useful in building a quantitative understanding of bilayer heterogeneity and in the development of relationships to predict the relative partitioning and transport of simple solutes into and across bilayers, most drug molecules of interest are structurally more complex. They may possess one or more polar functional groups along with nonpolar domains that provide substantial hydrophobic surface area and result in significant amphiphilic character. The polar functional groups may be well-isolated or in close proximity to each other and capable of intramolecular interactions. Such amphiphilic molecules are likely to adopt preferred orientations and, with sufficient conformational flexibility, preferred configurations that maximize favorable interactions with both polar and nonpolar regions of the lipid bilayer. Finally, even drug molecules having molecular weights well below 500 may nevertheless span a significant fraction of the total bilayer thickness in their preferred orientation and thus may exist within more than a single region within the bilayer.

In a recent investigation in this laboratory,[14] MD simulations were conducted for a structurally related set of compounds (tyramine, phenethylamine, and 4-ethylphenol) in DOPC bilayers to generate free energy profiles and functional group contributions to their free energies of transfer from water to various locations in the bilayer. The results obtained were compared to parallel experimental data that had been previously generated for the same compounds.[5] MD simulations for these solutes indicated the simultaneous existence of a barrier domain (i.e., a high free energy of transfer or low partition coefficient) near the bilayer center and a preferred binding region (i.e., low free energy of transfer or high partition coefficient) at the bilayer interface.

Here we report details of the interatomic interactions between these solutes and the phospholipids or water molecules within the bilayer and the importance of the solute's orientations relative to the lipid bilayer that give rise to those free energy profiles. These investigations provide significant insights regarding the molecular forces underlying the position-dependent free energy profiles obtained from the MD simulations.

The idea that asymmetric molecules will orient at an interface is very well accepted in the study of interfacial phenomena[15] and is obviously the underlying mechanism for the structure of the bilayer itself. Since lipid bilayers contain regions of varying chemical environments, solute molecules present in the lipid bilayer are likely to demonstrate preferred orientation at the interfaces of these environments. In the present work, the orientation of each solute relative to the bilayer during the simulation run has been monitored using a novel method to explore: (1) the prevalence of preferred orientations in various regions of the bilayer; (2) the potential correlation of intermolecular interactions to these preferred orientations; and (3) the impact of multiple polar functional groups versus a single functional group on the free energy profiles and orientational preferences.

Finally, the role of water molecules within the lipid bilayer and their contribution to the overall solvation of the solutes was explored. Water incursions in the form of water wires into the lipid bilayer membrane have been reported in previous MD studies,[16−18] but their importance is not yet fully understood.

METHODS

Systems for MD Simulations. Four atomic-level MD simulations in 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) bilayers were described previously.[14] These were conducted either with multiple copies of one of three solutes (tyramine, phenethylamine, or 4-ethylphenol) or, as a control, without any solutes at all. Each fully hydrated lipid bilayer system contained all atoms of 72 DOPC molecules and at least 3000 water molecules within a simulation cell constructed with periodic boundary conditions simulation box that was 83.5 Å normal to the bilayer and 50.9 Å long in each of the two dimensions of the plane of the bilayer.

Nineteen copies of the solute molecules were included, partly since this corresponded to the concentration range used in the experiments (0.16 M within the bilayer), which were designed in tandem with the simulations, and partly to achieve sufficient sampling while keeping the simulation runs to a reasonable length. These molecules were evenly distributed throughout the entire simulation box in all three dimensions, in the water as well as within the bilayer itself (which contained nine solutes) with sufficient spacing to avoid interactions between solute molecules so that the simulations mimicked the dilute conditions employed in experiments. The average distance between any given solute and its nearest neighbor was 14 Å. As previously reported, experimentally determined structural properties of the bilayer were monitored throughout the simulations (e.g., order parameters, bilayer thickness, overall density, density profile, and atom distribution profiles). Interestingly, no apparent impact of the solute molecules on the lipid bilayer was detected when compared to the simulation of the control bilayer which was devoid of solute. The reason for this may be that the total volume of the 19 solutes is quite small, slightly over 1% of the volume of the simulation box (the largest molecule, tyramine, has a volume of 135 Å^3).

Force Fields and Simulation Details. A modified version of the consistent valence force field (CVFF) described previously[19−21] was employed. The model used for water was a flexible three-center simple point charge (SPC)-like model, which has been found to generate properties for water that are in good agreement with those calculated using other water potentials[21,22] and with experimental data. Partial atomic charges needed for the solute molecules were computed using Jaguar 6.0[23] by employing an HF-SCF type calculation carried out using the 6-31G* basis set compatible with that used to calculate the charges for the rest of the system. A single point calculation was carried out for a geometry-optimized structure that allowed calculation of the molecular electrostatic potentials (ESPs). The ESP near the van der Waals surface was subsequently used to derive point charges at atomic centers.

As noted in our previous work, the MD simulations were run using LAMMPS 2001[24,25] operated on an SGI Origin 3000 computer where the number of CPUs varied from 4 to 16 depending on availability. A time step of 1 fs was used, without constraints on any bond lengths, within the velocity Verlet algorithm to integrate Newton's equations of motion. The Nose−Hoover thermostat implemented in LAMMPS 2001 was used to control the temperature of the system of the NVT simulations at 298 K where the DOPC bilayer is in a liquid crystalline state. The electrostatics were computed using the

particle−particle particle-mesh (PPPM) method[26] with a cutoff of 10 Å.

For each of the above-mentioned simulation systems several parallel simulations starting at different conformations were run to enhance sampling. In total over 200 ns were simulated, providing on average almost 1 μs of sampling (50 ns × 19 solutes) for each solute.

Convergence was determined through continual monitoring of the bilayer structural and conformational properties mentioned above and solute rotational properties, as we will describe. We carefully monitored the relative errors of the computed transfer free energies which decreased to fluctuations on the order of hundredths of a kcal/mol after 25−30 ns, after which the simulations continued for several tens of nanoseconds.

Construction of the Position-Dependent Free Energy Profiles. The free energy profiles were described previously[14] and are not the focus of the current manuscript. However, since they are an important part of the analyses, we repeat our procedure here.

Two approaches were used. For water molecules that were numerous and unconstrained during the simulations, a population density method was employed. Since the solutes were less numerous and the unrestrained solute molecules would tend to concentrate at low energy locations within the bilayer thus limiting the information obtainable for locations where energy barriers exist, an umbrella sampling technique[27−30] was employed to constrain the solute molecules at various locations to provide adequate coverage of sampling across the entire bilayer. Both methods are described only briefly as detailed descriptions are common in the literature.

1. Population Density Method. Since the reaction coordinate of interest is the bilayer normal, water molecule locations were defined relative to the central plane of the bilayer. The location of the central plane of the bilayer was calculated at each time step as the average of the locations of the phosphorus atoms of the lipid molecules in each of the two monolayers. The resulting water locations were then used to generate the population density along the bilayer normal. If $p(z)$ is the probability distribution of the water molecules along the bilayer depth, z, the corresponding free energy profile along the reaction coordinate is given as

$$\Delta G(z) = -k_B T \ln(p(z)) + C \quad (1)$$

where k_B is the Boltzmann constant, T is absolute temperature, $\Delta G(z)$ is the reversible work required to move a water molecule from the reference location (in bulk water) to a location corresponding to a point z in the bilayer, and C is a constant related to the choice of the reference location (i.e., if a location (z_w) in bulk water is chosen as the point of reference, the value of C is calculated as $k_B T \ln(p(z_w))$. The probability distribution of the unconstrained water molecules along the bilayer depth resulting from random thermal motions allows the computation of the free energy profile using the above equation.

This method is particularly useful for very small solutes and has been used to define the distribution of water across a lipid bilayer by Marrink and Berendsen.[3] While they found that this method was only partially useful, the length of simulations in the present study (3000 water molecules with tens of nanoseconds of trajectories amounting to almost 10 μs of total trajectory) allowed sufficient sampling for this calculation to succeed.

2. Force Integration Method. This method utilizes the quadratic constraint described above along with the force integration method described previously in the literature.[3] The quadratic potential was applied to the aromatic carbon attached to the ethyl group (i.e., the atom nearest to the center of mass) of each solute during the MD simulations to impose a "restoring" force only in the direction perpendicular to the bilayer plane to restrain the solutes in multiple broad regions. Lateral movement in the plane of the bilayer and rotation of the solutes were not restricted. After an initial equilibration phase of 2 ns during which the data were discarded, simulation results were recorded every picosecond. The accumulated data were subsequently used as described previously[14] to construct position-dependent free energy profiles for each solute. Since the copies of the solutes were widely distributed throughout the box, copies of solute molecules very rarely approached each other; this happened only twice during the simulation. When any two atoms of two individual solute molecules came within 5 Å of each other, those configurations were not included in the analysis to prevent possible artifacts resulting from solute−solute interactions on the observed profiles. However, one might argue that, given the experimental concentrations to which these profiles are compared, solute/solute interactions would not be unphysical.

Force constants employed during the production runs were optimized using trial runs with the objective of minimizing the constraint on solute motions while obtaining sufficient sampling at all locations. Force constants used for the restraining potentials in the production runs ranged from 0.1 to 1 kcal/mol·Å^2 depending on the location of solute copy within the bilayer. The restraint information was retained with the rest of the simulation data throughout the run. Since the reaction coordinate is the location along the bilayer normal, only the component of the force along this direction was used in all of the calculations. The net displacement force on each solute molecule was determined by adding together the forces on each of the constituent atoms as determined at each time step by the MD simulator. The component of this force along the bilayer normal was then corrected for the applied constraint force (in the same direction) by subtraction. The resulting quantity was taken as the force exerted along the normal by the local chemical environment on the solute and was recorded as a function of time.

The location of each solute at each time step relative to the central plane of the bilayer was also calculated and stored as a function of simulation time along with the net forces. For each 0.3 Å (we previously reported our optimization of this quantity[14]) slice of the simulation box, the force exerted on the solute across the entire simulation length was averaged. The average forces thus obtained were used to obtain the free energy profiles as described previously.[14]

The results of the constrained simulations were analyzed using a force integration method to obtain position-dependent free energy profiles for each of the three solutes.[14] We originally explored the use of the general technique of which WHAM is one implementation, but as others have noted, it has its drawbacks (e.g., ref 31). In particular the process involves subtracting an average biasing energy from the logarithm of the probability density for a particular window. This sets at odds the desire to have a larger window to improve the statistics against the desire to have a smaller window to decrease the error in assuming a constant biasing energy for the bin. In short, the statistics are often nonoptimal. By instead looking at

the instantaneous forces, one can exactly (and instantaneously) subtract the biasing force, and thus one can focus the "windowing" to optimize the statistics of the "real" force average at a given location. This also allowed us to use the smallest possible restraining forces while still sampling low probability locations. Small restraining forces allow for maximum translational and rotational movement of the solutes and reduce possible artifacts arising from restraints.

Hydrogen-Bond Interactions. Hydrogen bonds were searched and tabulated for the entire trajectory for each polar group on all copies of solute in each simulation system. The distances of the oxygen atoms of water or lipid functional groups from the heavy atom of the polar functional group of the solute (N or O) were calculated. If the distance was found to be less than 3.2 Å,[32] the interaction was defined as a hydrogen bond and was subsequently tabulated appropriately based on the solute location and the type of functional group to which it was bound. The counts for each hydrogen-bond type in each 0.3 Å slice of the simulation box were accumulated along with the average distance between the heavy atoms to calculate the average bond length. It should be noted that the solute locations refer to the locations of the aromatic carbon attached to the ethyl chain, whereas the polar functional groups are located at the extremes of the solute molecules. Therefore, the actual hydrogen bond may be located up to 5 Å away from the location indicated.

Solute Orientation with Respect to the Bilayer Normal. *1. Calculation of the Orientation Angles.* The angle made by the unit longitudinal vector of the solute with a unit normal vector (a unit vector starting at the central plane of the bilayer and perpendicular to the plane) was calculated for each solute copy at each time step of the simulation. The angle of the longitudinal vector of the solute was set to zero when the bond between the aromatic carbon and the adjacent carbon of the ethyl chain to which it was attached pointed toward the central plane of the bilayer and the opposite carbon−hydrogen bond or carbon−oxygen bond on the same ring pointed toward water. Due to symmetry across the central plane of the bilayer, the coordinates of the solute copies present in the two leaflets were transformed from box coordinates to relative coordinates before the orientation angle was calculated. Solute copies present near the center were assigned to the leaflet in which the central atom of the solute (i.e., the aromatic atom of the solute molecule attached to the ethyl chain) resided.

2. Calculation of the Orientation Angle Probability. Assume that the unit normal vector and the unit longitudinal vector of the solute are present along the radius (R) of a sphere with their tails at the center and the heads at the surface. The probability of occurrence of an angle of 0° to θ° between the two vectors would be proportional to the surface area ($= 2\pi Rh$) of the spherical cap having a height h ($h = R - R\cos\theta$) that is specified by the rotation of the solute vector around the normal vector (Figure 1).[33] Since both the solute longitudinal vector and the bilayer normal vector are unit vectors, the value of R is 1. The probability of the occurrence of the angle is then given as

$$P(\Theta) = 1 - \cos\Theta \quad (2)$$

and the probability of the occurrence of an angle between θ_1 and θ_2 is given as

$$P(\Theta_2 - \Theta_1) = |\cos\Theta_1 - \cos\Theta_2| \quad (3)$$

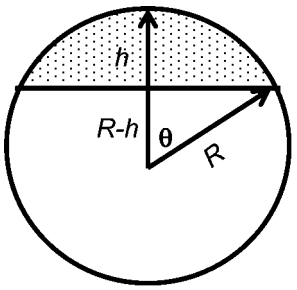

Figure 1. Unit normal vector (vertical arrow) and the unit longitudinal vector of the solute (arrow with label R) at an angle θ. R is the radius of the sphere, and the spherical cap specified by the rotation of the solute vector around the normal vector is shown with dots.

Normalizing to the entire possible range of 0−180° angles it can be shown that in an isotropic medium there is an equal probability of occurrence (20%) for each of the following five windows of angles: 0−53.1° (approximately perpendicular to the plane of the bilayer), 53.1−78.44° (tilted perpendicular), 78.44−101.51° (parallel to the plane of the bilayer), 101.51−126.84° (tilted inverse perpendicular), and 126.84−180° (inverse perpendicular). For ease of presentation, the solute orientations were tabulated according to these criteria to obtain counts of occurrence in these five classes that would have equal probability of occurrence in an isotropic environment such as bulk water.

RESULTS

Free Energy Profiles. The free energies of transfer for each of the three solutes from water to various bilayer locations were reproduced and further annotated from our previous work and are shown in Figure 2 (upper panel) along with the locations of the lipid phosphorus atoms, nitrogen atoms, carbonyl groups, and water molecules (lower panel). The water distribution profile was reduced by six-fold for the purposes of this plot. As previously discussed, all three solutes exhibit a preference for binding at the membrane interface with the minimum in free energy (approximately −5 to −6 kcal/mol relative to water) nearly superimposable with the location of the carbonyl groups of the ester linkages and a free energy maximum (approximately −3 to 2 kcal/mol) located at the center of the bilayer.

To investigate possible effects of the presence of the solutes on water incorporation into the bilayer, position-dependent free energy profiles of water calculated from each of the three simulation systems containing 4-ethylphenol, phenethylamine, or tyramine are shown in Figure 3. The profile of water in the absence of added solutes was redrawn from a simulation of a dipalmitoylphosphatidylcholine (DPPC) bilayer previously published by Shinoda et al.[34] In all profiles the free energy increases monotonically from the bulk water phase to the center of the bilayer.

Hydrogen-Bond Interactions. The average number of hydrogen bonds formed by the −OH or $-NH_2$ polar functional groups of the molecules explored in this study are shown as a function of location in the lipid bilayer in Figure 4 (where the location is determined by the position of the aromatic carbon attached to the ethyl chain). The configurations showing all hydrogen bonds, those formed with water, and those formed with the lipid molecules are shown as separate curves in the above figure. (Note that, because the locations specified are those of only the one central atom of the molecule as described

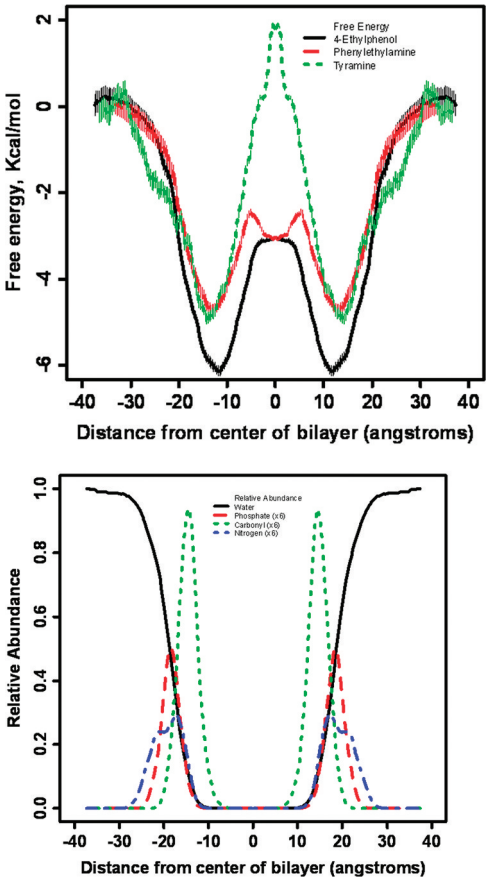

Figure 2. Position-dependent free energy profiles of tyramine, phenylethylamine, and 4-ethylphenol (upper panel) and position-dependent distributions of various atoms, molecules, or functional groups in the lipid bilayer (lower panel). The errors in the estimation of the free energy are shown by the vertical bars on each points of the curves. The distribution for the water molecules was reduced by six-fold for plotting purposes. The data were averaged over the two monolayers of the bilayer assuming bilayer symmetry and plotted as two halves for clarity of representation.

previously, the actual hydrogen bond may be located up to 5 Å away from the location indicated in the plot.) For each functional group, the average number of hydrogen bonds reaches a maximum value in bulk water. In general, the phenolic −OH groups of tyramine form a greater number of hydrogen bonds on average than the $-NH_2$ groups in tyramine and phenethylamine as seen by comparing the two left-hand figures with the right-hand figures in Figure 4.

Hydrogen bonding of the solute with water predominated over hydrogen bonds involving polar atoms of the phospholipid head groups in each of the regions, including the hydrocarbon interior. The solute−water hydrogen-bond lengths were reproducible in a given environment but slightly shorter (<0.1 Å) in the bilayer interior compared to those in bulk water.

While the majority of the hydrogen bonds involving solute are formed with water, the remaining hydrogen bonds are formed with polar phospholipid atoms located in the head-group region or further into the bilayer interior. The bottom panel of Figure 4 shows the average number of hydrogen bonds formed by 4-ethylphenol with various types of functional groups as a function of depth within the bilayer. Among these, hydrogen bonds with the phosphoryl group (P═O) outnumber all others in

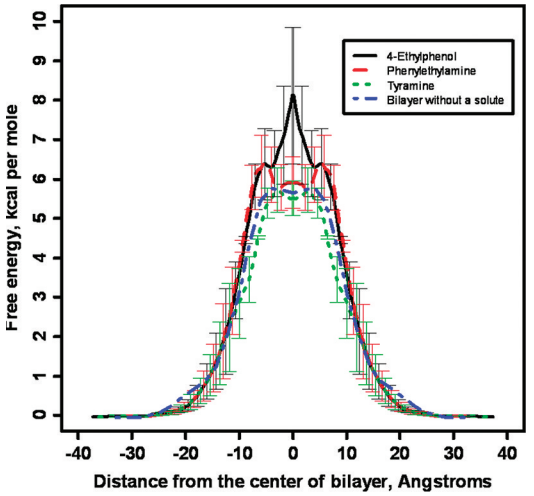

Figure 3. Free energy of transfer of water from bulk water to various locations within the DOPC bilayer. The profiles were obtained from the MD simulations of the lipid bilayer containing either 4-ethylphenol, phenylethylamine, or tyramine. An additional profile for the free energy of transfer of water into a DPPC bilayer in the absences of solute has been redrawn from the work of Shinoda et al.[34] The data were averaged over the two monolayers assuming bilayer symmetry and plotted as two halves for clarity of representation.

the head-group region, whereas those involving carbonyls outnumber all others in the bilayer interior. As a general trend, the ether oxygen atoms of the phospholipid form fewer hydrogen bonds with the solutes than the more exposed phosphoryl (P═O) or carbonyl groups (C═O). The phenolic −OH groups in 4-ethylphenol and tyramine form approximately 10 times more hydrogen bonds with the phosphoryl oxygen atoms (P═O) in the head-group region than with the phosphoether oxygen atoms (P−O). The $-NH_2$ groups of phenethylamine and tyramine do not form a sufficient number of hydrogen bonds with either type of oxygen atom of the head-group region to clearly establish a trend.

Solute Orientation with Respect to the Bilayer. The orientation of each solute copy was measured in reference to the vector perpendicular to the plane of the bilayer (the normal) having its origin at the center of the bilayer and pointing toward the head groups. The angle made by the solute longitudinal vector (head at the benzene ring and origin at the ethyl chain) with the bilayer normal was recorded as the orientation angle. The fractions of solute orientations found in each of the following five windows classes: 0−53.1° (perpendicular to the plane of the bilayer), 53.1−78.44° (tilted perpendicular), 78.44−101.51° (parallel to the plane of the bilayer), 101.51−126.84° (tilted inverse perpendicular), and 126.84−180° (inverse perpendicular) are shown as a function of distance from the center of the bilayer in Figures 5, 6, and 7 for 4-ethylphenol, phenethylamine, and tyramine, respectively. Only one leaflet of the bilayer is shown in each figure with the center of the bilayer at the left and the water region on the right-hand side.

As expected, all three solutes are oriented isotropically in bulk water. Deviations from equal proportions in water occur at a distance of ~30 Å from the center of the bilayer, which is approximately 10 Å from the location of the head groups as defined by the location of the phosphorus atoms. All three solutes approach the lipid bilayer from bulk water preferably with their ethyl chains pointing away from the bilayer, that is, in

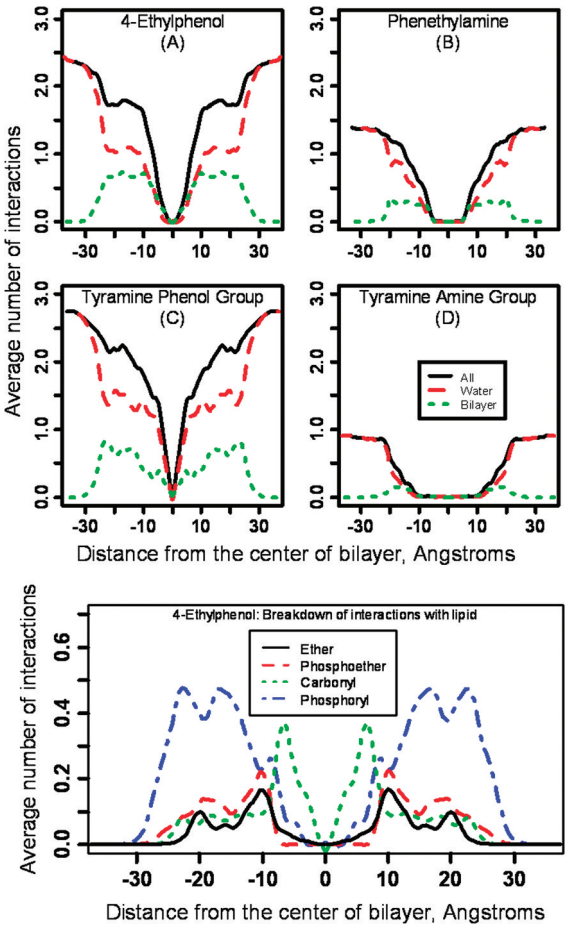

Figure 4. Upper panels: Average number of hydrogen-bond interactions formed by the three solutes as a function of location in the DOPC lipid bilayer. The location shown on the abscissa is that of the phenyl carbon attached to the ethyl chain (the actual location of the hydrogen bond may be 2−5 Å on either side). Lower panel: Average number of hydrogen bonds formed by 4-ethylphenol with various types of functional groups as a function of depth within the bilayer. The data were averaged over the two monolayers assuming bilayer symmetry and plotted as two halves for clarity of representation.

the inverse perpendicular orientation (at an angle of about 180°). Other similar orientations (i.e., with the ethyl chains at an angle pointing away from the bilayer surface) also occur in significant proportions in this region. These preferred orientations occur at the expense of both opposite orientations and orientations parallel to the bilayer plane.

All three solutes exhibit an increase in the parallel (78.44−101.51°) orientations near the head-group region (20 Å from the center of the bilayer) close to the average location of phosphorus atoms of the lipid bilayer. These orientations reach a maximum probability slightly on the inside of the interface for 4-ethylphenol, whereas for phenethylamine and tyramine the peaks coincide with the average location of the head groups (~20 Å from the center). In the case of tyramine parallel (78.44−101.51°) orientations are the most abundant within a narrow region at approximately 20 Å from the bilayer center.

On the inside of the head groups at approximately 15 Å from the bilayer center and close to the average location of the phospholipid carbonyls, tyramine and 4-ethylphenol show a strong preference for orientations in which their −OH groups

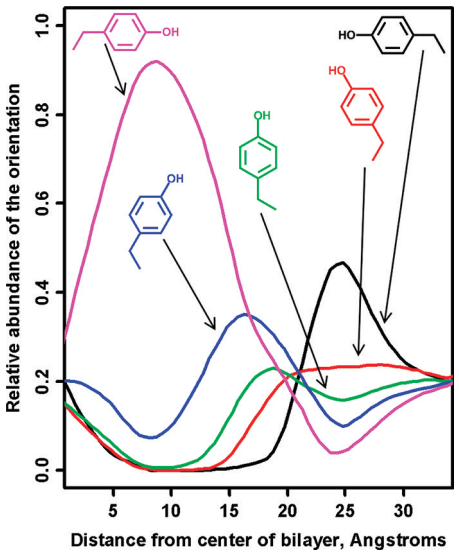

Figure 5. Relative distribution of orientations of 4-ethylphenol as a function of distance from the center of the bilayer.

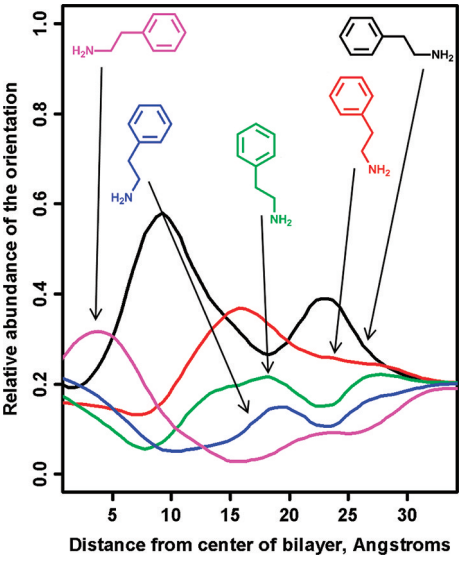

Figure 6. Relative distribution of orientations of phenylethylamine as a function of distance from the center of the bilayer.

point toward the lipid head groups, whereas phenethylamine exhibits a strong preference for the inverse perpendicular (126.84−180°) orientation in which the $-NH_2$ is pointed toward the lipid head groups to retain hydrogen-bonding interactions with interpenetrating water molecules or lipid carbonyls. These trends continue further into the ordered chain region, where the extent of the preference for orientations in which the −OH is pointed toward the head-group region peaks at 90% for 4-ethylphenol and tyramine. In the same location, the preference of phenethylamine for the inverse perpendicular (126.84−180°) orientation is only 60%. Near the center of the bilayer, the solutes are again isotropically distributed, suggesting free molecular rotation.

DISCUSSION

Free Energy Profiles. Solutes in this and other recently reported MD simulation studies can be grouped into at least three different classes based on their position-dependent free

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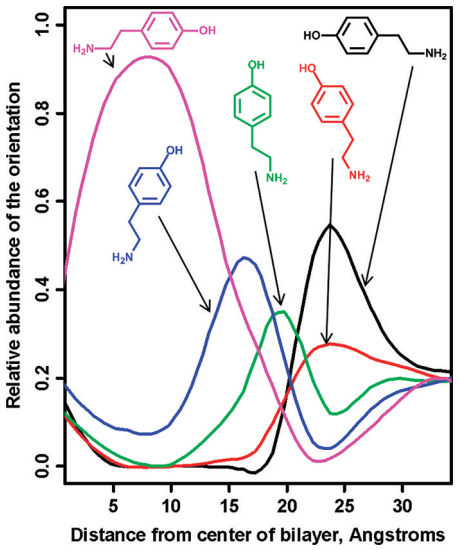

Figure 7. Relative distribution of orientations of tyramine as a function of distance from the center of the bilayer.

energy profiles: small hydrophobic solutes, small polar solutes, and amphiphilic molecules. Small hydrophobic solutes (e.g., methane, ethane, hexane, benzene, etc.) localize preferentially in the bilayer center. These profiles have been rationalized based on the classical hydrophobic effect which results in low solubility in water and high solubility in the acyl chain region of bilayers. Typically, partitioning of hydrophobic solutes into the dense and highly charged head-group region is less preferred relative to water.[10,13,32] Small hydrophobic solutes exhibit a relatively gradual increase in their concentration with depth going from the ordered chain region to the center of the bilayer. The increasing solute size is accompanied by a steeper gradient toward the bilayer center. This exclusion of hydrophobic solutes from the ordered chain region has been referred to as the nonclassical hydrophobic effect.[4,35−39] Examples of free energy profiles for the hydrophobic solutes methane, ethane, and hexane[13,32,47] are shown in Figure 8. Small polar

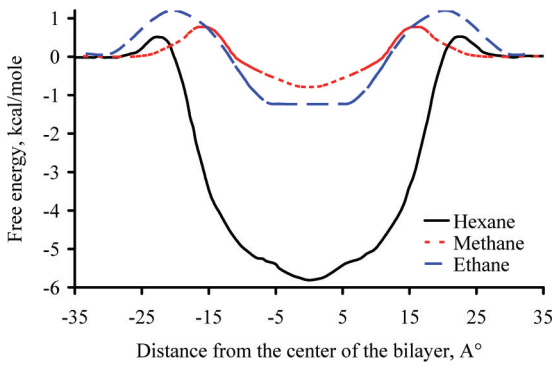

Figure 8. Position-dependent free energy profiles of hexane and ethane in a DOPC bilayer and the profile of methane in a dimyristoylphosphatidylcholine (DMPC) bilayer (redrawn from MacCallum and Tieleman,[47] Bemporad et al.,[13] and Stouch,[32] respectively).

solutes on the other hand may show opposite profiles with a free energy maximum, rather than a minimum, in the hydrocarbon region. An often-studied example of this is the profile of water[3,13,34] (Figure 3).

Amphiphilic solutes, such as those studied here, having both polar and nonpolar regions exhibit position-dependent free energy profiles that reflect their complexity. The profiles in general show a decrease in free energy (relative to bulk water) at or near the interface between the hydrophobic and the hydrophilic regions followed by an increase in the free energy (relative to this interface and/or to the bulk water) at the center of the bilayer. Profiles for tyramine, phenethylamine, and 4-ethylphenol shown in Figure 2 correspond to this type. Additional examples of profiles showing a preferred binding domain in the head-group region include adamantane derivatives,[40,41] the camptothecin analogue DB-67,[42] and several others.[8,43] Most of these studies attribute the preference for the interfacial region to the simultaneous solvation of the hydrophobic and hydrophilic regions of solute in the corresponding two adjacent regions of the bilayer (i.e., amphiphilic complementarity). A closer analysis of this region in all studies reporting position dependent free energy profiles reveals that, while the maximum partition coefficient occurs near the carbonyl groups, reductions in free energy begin about 10−15 Å further away from this region, while the solute is for the most part still in bulk water. This highlights the importance of the hydrophobic effect as the primary driving force for binding to the interfacial region.

Role of Intermolecular Interactions and Solute Orientations. A variety of shapes of free energy profiles for each type of solute discussed above are likely due to the fact that each solute has its own characteristic interactions with the lipid bilayer components. The specific interactions of each of the solutes in this study with the bilayer components and the solute orientations at various bilayer locations are discussed below. The discussion is organized according to location from the bilayer center, beginning in bulk water.

1. Bulk Water−Head-Group Interface. In bulk water at a distance of greater than 30 Å from the center of the bilayer, the number of hydrogen bonds for each solute is at its maximum compared with other regions of the bilayer (Figure 4). None of the solutes exhibit any orientational preferences. At 20−28 Å from the bilayer center (at the fringe of the bilayer water interface), the lipid choline groups appear to shield the hydrophobic parts of the solutes from the surrounding water. An example is shown in Figure 9 where the benzene ring of the

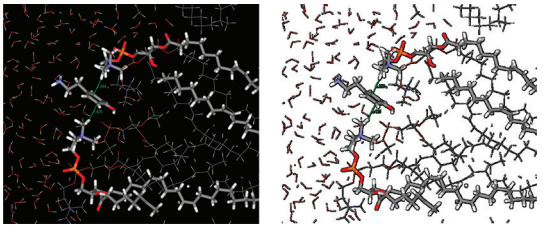

Figure 9. Snapshot of a tyramine molecule located on the water side of the water/head-group interface, viewed from inside the bilayer. Each side of the benzene ring is close to choline methyl groups as if it were solvated by the latter in this relatively polar environment. The phenol group is close to a phosphoryl group (which has been removed for visibility).

solute is shielded from the surrounding water molecules by choline methyl groups present nearby. Further support for this hypothesis comes from the fact that, irrespective of the presence of a polar group on the benzene ring or the ethyl chain, all three solutes prefer the inverse perpendicular (126.84−180°)

orientation in this region with the benzene ring pointing toward the head groups as shown in Figures 5, 6, and 7.

The ratio of populations of the favored to disfavored orientations can be used to estimate their relative free energies based on the Boltzmann equation.[44] For example, the preferred orientation of 4-ethylphenol and tyramine in this region is about 10-fold more favorable than the opposite orientation, corresponding to an energy difference of nearly 1.4 kcal/mol. The same ratio for phenethylamine in this region is about 4, which corresponds to approximately 0.8 kcal/mol. Even though solutes are still substantially solvated by water at this location, all three free energy profiles in Figure 2 display reductions of approximately 1 kcal/mol relative to bulk water. The decrease in free energy continues as the solutes approach the head-group region.

2. Head-Group Region. All three solutes approach the lipid bilayer with the phenyl group pointing toward it irrespective of the presence of a polar functional group on the aromatic ring. The decrease in the free energy associated with the solvation of hydrophobic components by the choline methyl functional groups culminates at a location slightly inside of the average head-group location near the carbonyl groups.

While it is conceivable that the strong hydrogen-bonding ability of head groups may contribute to the reduction in solute free energy in the head-group region, the average number of hydrogen bonds formed by the solute in the head-group region decreases slightly in comparison to the average in bulk water. While the average number of hydrogen bonds between the solute and the water decreases significantly in this region, it is partially compensated by an increase in solute hydrogen bonding with bilayer components. Additionally, an examination of the hydrogen-bond counts by the head-group type suggests that none of the head-group oxygen atoms form as many hydrogen bonds with any of the solutes as do the water molecules in this region. This is consistent with the fact that the number of water molecules present in this region exceeds the number of lipid molecules, so the opportunity for the solute to hydrogen bond with water molecules remains relatively high. Only in the vicinity of the carbonyl groups and beyond do polar atoms in the phospholipids become dominant (Figure 2). Consequently hydrogen-bond interactions are less likely to be the driving force accounting for the lowering of the free energy (or increase of partition coefficient) of these solutes in this region relative to that in the water.

At 20 Å from the center of the bilayer, at the approximate location of the phosphorus and nitrogen atoms of the head groups, orientations of 4-ethylphenol and tyramine parallel to the plane of the bilayer increase (Figures 5 and 7), yet the free energies for both solutes continue to decline monotonically (Figure 2). For the former, all orientations are nearly equally probable at approximately 20%, while for tyramine, the 90° orientation (parallel to the plane of bilayer) exhibits about a 10% advantage over all others (Figure 7). This orientation provides ample opportunities for both polar groups of tyramine to form hydrogen bonds with the polar atoms in the head groups or water. It is in this structured and dense region[45,46] (near 20 Å from the center of the bilayer) that hexane, butane, ethane, and benzene exhibit maxima in their free energies (Figure 8) of 0.5−1 kcal[8,16,47] that has been attributed to the lack of free volume.[47] The reorientation of the amphiphiles places their polar groups in the hydrogen-bonding network of the head groups rather than the hydrogen-bond disrupting hydrophobic groups. As a result of this compensating effect, the free energy profiles of the three solutes show no peak at this location, just a monotonic decline in free energy relative to water as a function of their distance away from bulk water until a minimum occurs at 10−15 Å.

3. Head-Group−Lipid Chain Interface. Near the minimum in the free energy profile (i.e., in the preferred binding region 15 Å from the center of the bilayer), all three solutes display almost equal proportions of two orientations (one perpendicular to the plane of the bilayer and the other a tilted version of the same), both of which orient a polar group toward the head group to preserve hydrogen bonding. Both orientations for each solute are conducive to the amphiphilic complementarity with the environment that is frequently cited as a reason for high partition coefficients in this region.[8,41−43,48] For tyramine, where two opposite orientations permit either −OH or $-NH_2$ hydrogen bonding with polar atoms in the phospholipids or water molecules within the head-group region, it is the orientation in which −OH is pointed toward the head groups that dominates. Shown in Figure 10 is a snapshot of

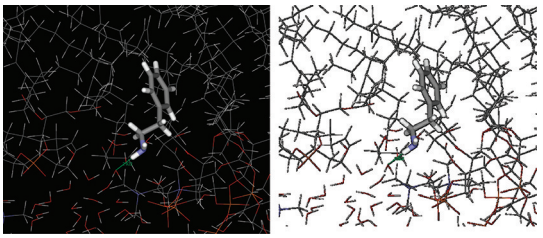

Figure 10. Snapshot of a phenylethylamine molecule located within the DOPC bilayer on the head-group side of the water/head-group interface where it forms a hydrogen bond (green line) with a nearby water molecule.

phenethylamine in this region oriented such that the $-NH_2$ group can form a hydrogen bond with a nearby water molecule, while the rest of the molecule remains in the lipid chain region. For all three solutes in this region, the minimum in the transfer free energy profile appears to reflect the combination of a favorable hydrophobic driving force coupled with the retention of the most favorable hydrogen-bonding interaction between the solute and either water molecules or polar lipid atoms.

4. Ordered Hydrocarbon Chain Region. The departure of the solute from the region of minimum free energy at 15 Å from the bilayer center into the ordered chain region within the bilayer interior is energetically disfavored. Likely, this is largely due to the progressive loss of hydrogen bonds, with the average number of hydrogen bonds per solute molecule approaching zero in the center of the bilayer (Figure 4) for all three solutes (it is conceivable that there is also an effect due to hydrocarbon chain ordering and density) This decline coincides with decreases both in carbonyl functional groups and water molecules through most of this region (from 15 to 5 Å from the center of bilayer).

Once the hydrogen-bonding contribution to the free energy has been removed, the solute is in a largely hydrocarbon-like environment, but the hydrogen-bonding contribution does not completely disappear until the solute is quite near the center of the bilayer. Presumably this is due to several factors, including the substantial size of the solute in relation to the bilayer thickness, the fact that the carbonyl oxygen concentration is not negligible even 10 Å from the bilayer center, and the

penetration of water into the interior. Water molecule incursions into lipid bilayers may be facilitated by water wire formation to penetrating solutes in some cases as shown in MD simulations by several laboratories.[16−18,42] Typical water wires of 2−3 water molecules solvating a solute in the bilayer interior are thought to last a few hundred picoseconds to nanoseconds and reappear fairly frequently. While the lifetime of such events was not monitored in this study, a review of the snapshots of the simulations did show such occurrences, as depicted in Figure 11, where a tyramine molecule located in the ordered

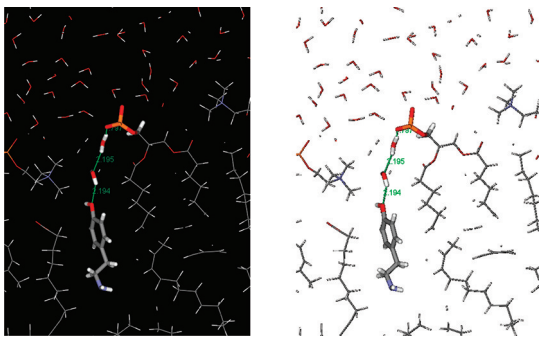

Figure 11. Snapshot of a tyramine molecule located at approximately 5 Å from the central plane of the bilayer. Green lines indicate hydrogen bonds within a water wire connecting a phosphoryl oxygen with the tyramine −OH.[56].

chain region 5 Å from the center of the bilayer is hydrogen-bonded to a water strand linked to a phosphoryl oxygen atom.

Preferred orientations of the solutes in this region are in accord with the retention of hydrogen bonding. For example about 90% of the populations of tyramine and 4-ethylphenol exhibit the perpendicular (0−53.1°) orientation that has a hydroxyl pointing toward head groups, and the rest of the orientations are slightly tilted versions of the same. The fraction of the inverse perpendicular (126.84−180°) orientation is virtually zero. Assuming a 0.1 to 0.01% probability for this orientation translates to an energy differential of about 4−5 kcal/mol. This free energy corresponds to the loss of a hydrogen bond for the hydroxyl group in going from the preferred orientation to the opposite and is comparable to the −OH group contribution to the free energy of solute transfer from water to a hydrocarbon solvent of 5.6 kcal/mol.[49]

Phenethylamine on the other hand is oriented preferentially (60%) in the inverse perpendicular (126.84−180°) orientation (with the $-NH_2$ pointing toward the head groups) with the tilted version of the same having about a 15% probability. The perpendicular (0−53.1°) orientation that is slightly preferred near the center (30%) is also seen at a 15% probability in this region. It is noteworthy that a sizable proportion of the phenethylamine population undergoes a complete flip between the ordered chain and central regions without displaying a significant fraction of the parallel (78.44−101.51°) orientation. This preference for either perpendicular orientation over the parallel is consistent with MD simulations of a neat lipid bilayer by Xiang and Anderson[50] which showed that the free volume in this region is elongated.

5. Center of the Bilayer. The chemical nature of the center of the bilayer is quite similar to that of a liquid hydrocarbon, and as a result relative partition coefficients in this region are correlated with hydrocarbon/water partition coefficients. The free energy differential for the amphiphilic solutes in this study is due to two opposing effects: the hydrophobic effect and the loss of hydrogen bonds to the polar functional groups, as all of the solutes show nearly zero hydrogen bonds in the center. The importance of this balance in determining the partitioning behavior of the compounds is reflected in the recent use of polar and nonpolar surface areas of the solute molecules in construction of predictive linear free energy relationship (LFER) equations.[51−53]

Orientational preferences diminish at the center of the bilayer (Figures 5−7) although there appears to be a bias toward the perpendicular (0−53.1°) orientations that have the aromatic ring pointing outward. This slight preference of the benzene ring to point away from the center could be due to interactions with the double bonds of the DOPC molecules providing some stabilization.

While rapid solute rotation was observed in bulk water, a question remains as to whether or not the solutes had an opportunity to sample all possible orientations in the ordered chain region. Since solutes were found to move in and out of the lipid bilayer slices (due to the very weak constraint force applied) it was difficult to construct correlation functions for their orientation in a given slice. However, another approach to understanding this is shown in Figure 12 which shows four

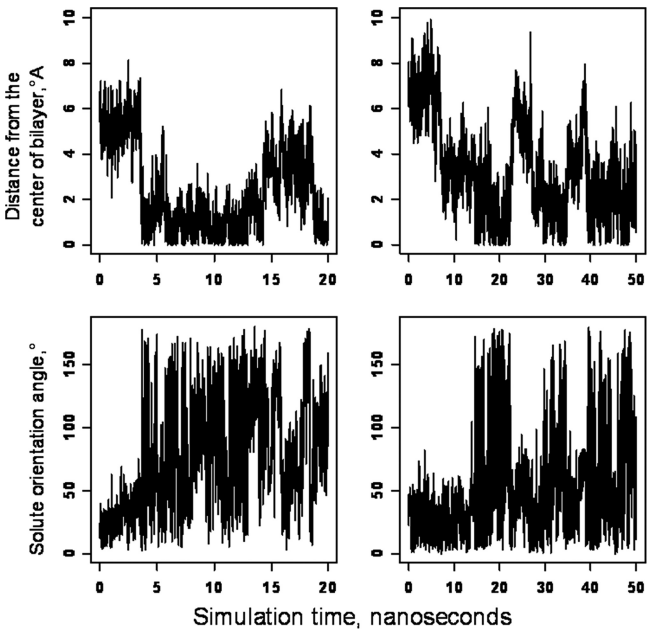

Figure 12. Two representative trajectories from the simulation of 4-ethylphenol. Top panels show the location of solute relative to the center of the bilayer versus the simulation time. Bottom panels show the corresponding orientation angle of the solute as a function of the same simulation times as shown in the upper panels.

panels: the top two show the location of the solute and the bottom two show the orientation, both as a function of time. The left pair represents one solute, and the right pair represents another. Looking at the top two panels alone, it is apparent that the solutes can move over distances as much as 10 Å despite the constraints applied to them. This verifies that the constraining potential employed was significantly weaker than the typical magnitude of forces exchanged during the course of MD simulation (it is truly a biasing potential) as discussed earlier. Looking at the left pair of panels, during the first few nanoseconds (0−5 ns) the solute adopts a preferred orientation

while it is located away from the center, but it rotates freely when it happens to move closer to the center of the bilayer. All orientations that occur near the center have equal probability and are free to relocate away from the bilayer center. However, repeatedly one finds a preferred orientation when the solute is located 4−10 Å from the bilayer center. The right pair of panels illustrates the same processes repeatedly over 50 ns. Figure 12 suggests that when the solutes are present in the center of the bilayer all possible orientations have significant probabilities and are sampled frequently, whereas solutes in the ordered chain region exhibit the preferred orientations discussed previously. Thus, there are sufficient opportunities for all solute orientations in the bilayer center to enter the ordered chain region but only a select few actually enter. The application of a weak quadratic constraint allowed sufficient freedom for the solute molecules to exit the low mobility regions, change orientations, and return thereby providing adequate sampling of all orientations at these locations.

Additivity or Nonadditivity of Functional Group Contributions to the Transfer Free Energy. Previously, bilayer transport experiments have demonstrated that the barrier domain (after correction for unstirred boundary layer effects) governing the passive permeation of solutes that contain at least one hydrogen-bonding functional group is hydrocarbon-like, as demonstrated by the close correlation between egg phosphatidylcholine or DOPC bilayer permeability coefficients and partition coefficients for the same solutes between water and hydrocarbon solvents such as 1,9-decadiene.[54,55] The present study shows that, for the solutes explored herein, this region must be very near the bilayer center, at least in the relatively disordered liquid crystalline DOPC bilayer. Given that free energies of transfer into this region reflect nearly complete elimination of hydrogen bonds to the polar functional groups on these solutes, it is expected that functional group additivity to the transfer free energy should hold, providing that the groups in question are well-isolated from other polar groups on the same molecule.

On the other hand, the contributions of the hydroxyl and amino groups to transfer free energies of the solutes of interest in this study from water to the interfacial region of DOPC were previously shown to be close to zero in both MD simulations and experimental measurements.[14] With the results of the present study, it is now clear that the nonadditivity of polar functional group contributions to the free energy of transfer from water to the bilayer interface is a consequence of the fact that only a slight reduction in the average number of hydrogen bonds per solute molecule accompanies this transfer. Even tyramine, with two polar functional groups located at opposite ends of the molecule, is capable of adopting orientations at the bilayer interface that optimize hydrophobic interactions while preserving hydrogen-bonding interactions. For this reason, multiple polar functional groups on the same solute are likely to impact the magnitude of the unfavorable free energy in the barrier region more than they affect the free energy in the preferred binding region near the lipid head groups.

■ CONCLUSION

MD simulations have been conducted in DOPC bilayers to examine the free energies of transfer of three structurally related drug-like molecules (4-ethylphenol, phenethylamine, and tyramine) from water into various regions of the bilayer and the molecular interactions leading to the free energy profiles observed. The larger size of the solutes, which can span a significant fraction of the bilayer thickness, allows them to adopt orientations that maximize favorable interactions within different bilayer regions. The number and types of hydrogen bonds formed between the three solutes and specific functionalities within the polar head groups and with water molecules were determined as a function of solute position in the bilayer. In all regions, hydrogen bonds with water molecules account for the majority of hydrogen-bonding interactions observed for each solute.

Previously, the location where the transfer free energy of the three solutes is highest, which defines the barrier domain for permeability, was found to be the bilayer center, while the interfacial region was found to be the preferred binding region. Contributions of the $-NH_2$ and $-OH$ functional groups to the transfer free energies from water to the interfacial region were found to be very small both experimentally and by MD simulation, suggesting that the interfacial binding of these solutes is hydrophobically driven and occurs with minimal losses in hydrogen-bonding interactions of the polar functional groups with either water or phospholipid head groups. Therefore, interfacial binding is relatively insensitive to the number or type of polar functional groups on the solute. Significant orientational preferences for the solutes were observed, with those that preserve hydrogen bonding being favored in the interfacial region.

Minima in the number of hydrogen bonds formed by each solute were observed at the center of the bilayer, coinciding with the decrease in the number of water molecules in DOPC as a function of distance away from the interfacial region. As a consequence, maxima in the free energy profiles were observed in this region, which were highly sensitive to the number of polar functional groups on the molecule.

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