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Comparative Exploration of Hydrogen Sulfide and Water Trans-Membrane Free Energy Surfaces via Orthogonal Space Tempering Free Energy Sampling

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Abstract

Hydrogen sulfide (H₂S), a commonly known toxic gas compound, possesses unique chemical features that allow this small solute molecule to quickly diffuse through cell membranes. Taking advantage of the recent orthogonal space tempering (OST) method, we comparatively mapped the trans-membrane (POPC) free energy landscapes of H₂S and its structural analogue, water (H₂O), seeking to decipher the molecular determinants that govern their drastically different permeabilities. As revealed by our OST sampling results, in contrast to the highly polar water solute, hydrogen sulfide is evidently amphipathic, and thus inside membrane is favorably localized at the interfacial region, i.e. the interface between the polar head-group and non-polar acyl chain regions. Because the membrane binding affinity of H₂S is mainly governed by its small hydrophobic moiety and the barrier height in between the interfacial region and the membrane center is largely determined by its moderate polarity, the trans-membrane free energy barriers to encounter by this toxic molecule are very small. Moreover when H₂S diffuses from the bulk solution to the membrane center, the above two effects nearly cancel each other, so as to lead to a negligible free energy difference. This study not only explains why H₂S can quickly pass through cell membranes but also provides a practical illustration on how to employ the OST free energy sampling method to conveniently analyze complex molecular processes.

Introduction

Understanding how chemical structures influence passive membrane permeability is essential to both membrane biophysical research and drug discovery process. Due to the fact

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that solute molecules may have complex interactions with different lipid bilayer regions, atomistic level studies are necessary even for seemingly simple molecules, such as hydrogen sulfide (H_2S). H_2S has been known as a toxic gas compound; interestingly, recent biological investigations^{1–3} are progressively establishing it as a molecule of importance to various physiological functions. Although structurally similar to water (H_2O), which has a low permeability coefficient (around 10^{-5} cm/s)⁴, H_2S possesses unique chemical features that allow this small solute molecule to quickly cross lipid bilayer barriers⁵. The permeability coefficient of H_2S is experimentally estimated⁵ to be above 0.5 cm/s; the large value indicates that this polar molecule, unexpectedly like small nonpolar solutes, does not require any protein facilitator to overcome membrane barriers. Obviously, key questions, such as how does H_2S interact with membrane lipids and what are the molecular determinants that govern the drastically different permeation behaviors of the H_2S and H_2O solutes, need to be answered.

To quantitatively analyze solute-membrane interactions, mapping trans-membrane free energy (TMFE) landscapes via molecular dynamics (MD) simulation methods^{6–12} can be a viable strategy. It is worth noting that previous computational efforts have been mostly carried out through traditional free energy calculation methods such as umbrella sampling (US)¹³ and thermodynamic integration (TI)^{14,15}. In the past years, the increasingly affordable MD propagation power allows these commonly employed free energy approaches to be more critically assessed. As suggested by long time-scale simulation results¹¹, achieving adequate sampling of TMFE surfaces, even for simple solute molecules, can be computationally demanding. Such sampling difficulty is largely led by the fact that slow structural responses, such as orientation adjustment of solute molecules, reorganization and relocation of surrounding phospholipids and waters, and even coupling of these motions, can be intimately associated with across-membrane diffusion dynamics. Obviously “importance sampling” treatment along the membrane normal “z” alone, as employed in US or TI simulations, cannot actively accelerate crossings of the barriers that are associated with these slow “response” dynamics. Facing such “hidden free energy barrier” challenge^{16,17}, in this study, an orthogonal space sampling method^{16–18}, which allows the motions along a target order parameter and of its strongly-coupled environments to be synchronously accelerated, was employed so as to more reliably sample the trans-membrane processes of the H_2S and H_2O solutes. It is noted that this is the first work that applies the orthogonal space tempering (OST)¹⁸ algorithm¹⁸ to sample molecular membrane permeations.

In our simulation model, a common zwitterionic lipid, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylcholine (POPC) was used to construct the membrane bi-layer model. We comparatively sampled the trans-membrane free energy surfaces of the H_2S and H_2O solutes. As indicated by our OST simulation results, in contrast to the highly polar water solute, hydrogen sulfide is noticeably amphipathic, and thus in the membrane is favorably localized at the interfacial region, i.e. at the interface between the polar head-group and non-polar acyl chain regions. Because the membrane binding affinity of H_2S is mainly governed by its small hydrophobic moiety and the barrier height between the interfacial region and the membrane center is largely determined by its moderate polarity, the TMFE barriers experienced by this toxic molecule are all very small. Moreover when H_2S is transferred from the bulk solution to the membrane center, the above two effects nearly cancel each

other, leading to a negligible free energy difference. This study not only explains why H₂S can quickly pass through cell membranes but also provides a practical illustration on how to employ the OST free energy sampling method to reliably explore complex molecular events.

Computational Methods

In this section, the theoretical background, the simulation protocol, and the analysis method are briefly discussed; and the simulation setup is described.

The Orthogonal Space Tempering (OST) Free Energy Sampling Method

The OST algorithm was developed in the orthogonal space sampling scheme^{16–18}. To realize OST sampling on a trans-membrane permeation process, the original Hamiltonian H_o is modified to be

$$H_m = H_o + f_m(z(X)) + \alpha g_m(z(X), F_z), \quad (1)$$

where $z(X)$ denotes the membrane normal depth of the mass-weighted center of a target solute molecule, a commonly used order parameter for TMFE calculations; and α stands for a pre-set scaling parameter (between 0 and 1) that determines the F_z space sampling

aggressiveness (Note: the generalized force F_z is defined as $\frac{\partial H_o}{\partial z} - kT_o \frac{\partial \ln |J|}{\partial z}$, in which the latter term represents the Jacobian contribution; k stands for the Boltzmann constant and T_o denotes the system reservoir temperature). During an OST simulation, the first-order biasing potential $f_m(z(X))$ is recursively updated towards the negative of the z -dependent free energy profile, $-G_o(z)$ (under the “ H_o ” ensemble); and the second-order biasing potential $g_m(z(X), F_z)$ is adaptively updated towards the negative of the two-dimensional free energy profile along (z, F_z) , $-G_f(z, F_z)$ (under the “ $H_o - G_o(z)$ ” ensemble). In the OST method design, $f_m(z(X))$ is employed to remove z -dependent free energy barriers; and $\alpha g_m(z(X), F_z)$ is used to flatten the free energy surfaces along the generalized force F_z so as to promote strongly-coupled environment fluctuations. In the original OST method paper¹⁸, α was

expressed as $\frac{T_{ES} - T_o}{T_{ES}}$, where T_{ES} is called the orthogonal space sampling temperature

because at each z' state of the H_m ensemble, the F_z distribution is proportional to $e^{-\frac{G_f(z', F_z)}{kT_{ES}}}$.

The OST algorithm is a generalized ensemble free energy simulation technique. To realize adaptive generation of the biasing potential functions, an extended-dynamics based “double-integration” recursion strategy was developed¹⁸. Then, the target Hamiltonian (Equation 1) is further modified as follows:

$$H_m = H_\lambda + \frac{p_\phi^2}{2m_\phi} + \frac{1}{2} K_\phi \left(\frac{\partial H_\lambda}{\partial \lambda} - \phi \right)^2 + f_m(\lambda) + \alpha g_m(\lambda, \phi), \quad (2)$$

where $H_\lambda = H_o + \frac{p\lambda^2}{2m_\lambda} + \frac{1}{2}K_\lambda(z(X) - \theta(\lambda))^2$. Here, $\theta(\lambda)$ is defined as $z_{min} + \lambda(z_{max} - z_{min})$, in which z_{min} and z_{max} (respectively corresponding to the states of $\lambda = 0$ and $\lambda = 1$) represent the boundary values of the order parameter space. Treated as a fictitious particle with a mass of m_λ , λ is propagated based on an independent Langevin equation, the temperature in which is the same as the system reservoir temperature T_o . Through the restraint term

$\frac{1}{2}K_\lambda(z(X) - \theta(\lambda))^2$, $z(X)$ fluctuations and $\theta(\lambda)$ dynamics can be intimately synchronized. With the introduction of the λ -space extended-dynamics¹⁹, all the required sampling treatment can be focused on λ instead of the target order parameter $z(X)$, for instance, $f_m(z(X))$ in Equation 1 can be replaced by $f_m(\lambda)$, the recursion target of which is $-G_\lambda(\lambda)$, the negative of the λ -dependent free energy profile in the “ H_λ ” ensemble. Following the double-integration OST procedure, another extended-dynamics particle ϕ is introduced; and through

the energy term $\frac{1}{2}K_\phi(\frac{\partial H_\lambda}{\partial \lambda} - \phi)^2$, ϕ can act as the dynamic restraining reference for $\frac{\partial H_\lambda}{\partial \lambda}$. In correspondence, $g_m(z(X), F_z)$ in Equation 1 can be replaced by $g_m(\lambda, \phi)$, the recursion target of which is $-G_\phi(\lambda, \phi)$, the negative of the (λ, ϕ) space free energy profile under the

“ $H_\lambda - G_\lambda(\lambda) + \frac{1}{2}K_\phi(\frac{\partial H_\lambda}{\partial \lambda} - \phi)^2$ ” ensemble. The details on how to adaptively obtain $g_m(\lambda, \phi)$ and $f_m(\lambda)$ via a recursive double-integration protocol can be found out in the original OST method paper¹⁸. It should be noted that to restrain λ within the range between 0 and 1, a boundary potential

$$U_{\text{Bound}}(\lambda) = \begin{cases} \frac{1}{2}K_{\text{Bound}}\lambda^2, \lambda < 0 \\ 0, 0 \leq \lambda \leq 1 \\ \frac{1}{2}K_{\text{Bound}}(\lambda - 1)^2, \lambda > 1 \end{cases}, \quad (3)$$

is applied.

Orthogonal Space Tempering Result Analysis

When $g_m(\lambda, \phi)$ and $f_m(\lambda)$ recursions converge, the OST simulation naturally enters its equilibrium phase. Then, for any collective variable set $\vec{s}$ of interest, the probability distribution can be constructed based on the following equation,

$$\rho_o(\vec{s}) \propto \sum_t e^{-\frac{\frac{1}{2}K_\lambda(z(X(t)) - z(\lambda(t)))^2 + \frac{1}{2}K_\phi\left(\frac{\partial H_\lambda}{\partial \lambda}(t) - \phi(t)\right)^2 + f_m(\lambda(t)) + \alpha g_m(\lambda(t), \phi(t))}{kT_o}} \delta(\vec{s}(X(t)) - \vec{s}), \quad (4)$$

where t represents the scheduled sample-collecting time-steps in the equilibrium phase of the simulation. In correspondence, the $\vec{s}$ -dependent free energy surface can be generated based on

$$-kT_o \ln \left(\sum_t e^{-\frac{\frac{1}{2}K_\lambda(z(X(t)) - z(\lambda(t)))^2 + \frac{1}{2}K_\phi\left(\frac{\partial H_\lambda}{\partial \lambda}(t) - \phi(t)\right)^2 + f_m(\lambda(t)) + \alpha g_m(\lambda(t), \phi(t))}{kT_o}} \delta(\vec{s}(X(t)) - \vec{s}) \right).$$

The above extended-ensemble-to-canonical-ensemble re-weighting strategy has been employed in our earlier orthogonal space random walk practices^{17,20} and on-the-path random walk based simulation studies²¹. It is a generalized form of the umbrella sampling formula¹³, which was originally derived in the canonical-ensemble-to-canonical-ensemble reweighting framework.

The free energy profiles along the order parameter $z(X)$, $G_o(z)$, can be estimated through multiple means. The most straightforward way is to simply use $G_\lambda(\lambda)$, which is directly generated through on-the-fly recursions. Alternatively, based on Equation 4, one can directly obtain $\rho_o(z)$ and estimate $G_o(z)$ by $-kT_o \ln \rho_o(z)$. As shown in the section of *Results and Discussion*, these two methods lead to almost identical results; notably, the former method should converge faster for the fact that its convergence is less dependent on the near-equilibrium sample size.

The Model Setup

The membrane model building follows the procedure provided by the CHARMM-GUI server²². Each membrane model includes 64 POPC chains (32 in each leaflet), which are treated with the CHARMM potential²³; and the lipid-bilayer is aligned along the z axis and the solvated by 3765 water molecules, which are described with a modified TIP3P model²⁴. In correspondence, the starting size of the membrane construct is around $46.75 \text{ \AA} \times 46.75 \text{ \AA} \times 86 \text{ \AA}$ (XYZ). For each simulation, the solute molecule was first inserted into the bulk region; then the overall structure was equilibrated through a 70 pico-second (ps) conventional MD simulation.

The Simulation Setup

All the simulations in this study were performed in the NPAT ensemble, in which the x-y plane area was kept constant. The temperature was set as 303.15 K and the pressure along the membrane normal direction was set as 1 atm; the Nse-Hoover thermostat²⁵ was utilized to maintain the constant temperature and the Langevin piston algorithm²⁶ was employed to maintain the constant pressure. The particle mesh Ewald (PME) method²⁷ was used to treat long-range electrostatic interactions; in real space energy and force calculations, short-range electrostatic interactions were switched off at $10 \text{ \AA}$. The SHAKE algorithm was used to constrain all the bonds that involve hydrogen atoms. The MD simulation time step was set as 2 femto-seconds (fs).

The OST algorithm was implemented in our customized version of the CHARMM program^{28,29}. In this study, the z plane of the mass-weighted center of the lipid bilayer was used as the reference for the $z(X)$ definition. For the fact that a symmetric POPC lipid-bilayer model was employed, we set $z_{min}(-30 \text{ \AA})$ equal to $-z_{max}(30 \text{ \AA})$; i.e. when $\lambda = 0.5$, the solute molecules diffuse around the bi-layer center. Then, the TMFE profiles are expected to be symmetric. In our $z(X)$ -OST method implementation, a symmetry-enforcing strategy is applied; i.e. for any sample collected at the state of (λ, ϕ) , it is also simultaneously counted for the state of $((1 - \lambda), -\phi)$ with all the required quantities, such as $z(X)$ and $\frac{\partial H_\lambda}{\partial \lambda}$, recorded after necessary symmetry operations. Thereby, we can avoid setting

a boundary in the middle of the lipid bilayer; moreover, when the permeant crosses the bilayer center, the de-correlated lipid environment allows the free energy calculation to converge faster. It is noted that although the sampling space spans the whole bilayer, in this symmetry-enforcing scheme, indeed the target free energy profile range is only half of the overall range. In correspondence, when a solute starts from one end of the bulk solution (either $\lambda = 0$ or $\lambda = 1$) and reaches the membrane center ($\lambda = 0.5$) or vice versa, it can be counted as a complete one-way trip.

In the OST simulations of this study, the orthogonal space sampling temperature T_{ES} was set to 1500 K, corresponding to $\alpha = 0.8$; the masses of the fictitious particles m_λ and m_ϕ were respectively set as 10^3 atomic mass units (amu) and 10^{-5} amu; the restraint force constants K_z and K_ϕ were respectively set as $10 \text{ kcal}\cdot\text{mol}^{-1}\text{\AA}^{-2}$ and $5.0\times 10^{-5}\text{ kcal}\cdot\text{mol}^{-1}$. During OST recursions, both $f_m(\lambda)$ and $g_m(\lambda, \phi)$ were updated every 2 fs. The trajectories were saved every 1.0 pico-second (ps) for post-processing analysis.

Results and Discussion

In this study, we intend to explore hydrogen sulfide and water trans-membrane free energy landscapes and so seek to understand the molecular determinants that govern their significantly different permeation capabilities. The trans-membrane free energy change of the water solute has been previously studied and the z -dependent free energy profile obtained in this study agrees well with earlier results^{8,12}. Therefore, the following discussion on free energy convergence behaviors is mainly focused on the H_2S case.

Orthogonal Space Tempering Convergence Behaviors

As aforementioned, obtaining accurate TMFE profiles can be practically challenging. Here, we applied the OST method to enhance the sampling of solute passive membrane permeations. As shown in Figure 1a, the OST treatment allows H_2S to continuously travel between the bulk solution and the bilayer center. Within 90 nano-seconds (ns), fifteen one-way trips (between any end of the bulk solution and the bilayer center) were enabled. It is obvious that there are two major diffusion bottlenecks. They respectively correspond to the interface between the bulk solution and the membrane ($z = \pm 19\text{\AA}$) and the interface between the head-group region and the acyl-chain region of the bi-layer ($z = \pm 12\text{\AA}$).

To check sampling convergence, we first mapped the root mean square deviation (RMSD) between each pair of the free energy profiles, which were estimated at different time-steps (the inset of Figure 1b). Then, we monitored the time-dependent “maximum deviation value” (MDV) changes; at each checkpoint t' , MDV is defined as the maximum value at the upper right (t', t_{max}) corner of the RMSD map²¹. The MDV at time t' represents the largest uncertainty that the free energy surface $G_f(z)$ manifests itself within the overall simulation length t_{max} . Figure 1b shows that within 25 ns, MDV dropped below 0.5 kcal/mol and within 70 ns, MDV dropped below 0.1 kcal/mol. After 70 ns, three additional one-way trips occurred; this further strengthens our confidence on the quality of sampling convergence. Moreover, using the data between 70 ns and 90 ns, we calculated the MDV at each z location and plotted it as the corresponding free energy uncertainty. As shown in Figure 2a, larger uncertainties (around 0.1 kcal/mol or above) are distributed in the regions before H_2S enters

the bilayer ($z > 19\text{\AA}$ or $z < -19\text{\AA}$) and when it diffuses around the membrane center ($z = 0\text{\AA}$). As described in *Computational Methods*, we can utilize samples from the OST simulation to re-evaluate the free energy profile. Based on the MDV curve, we picked the samples collected in the last 40 ns. The TMFE surface derived from the post-processing umbrella re-weighting analysis (the gray curve in Figure 2a) agrees well with the TMFE surface generated from the OST recursion (the black curve in Figure 2a). Notably, the regions where two TMFE surfaces have larger discrepancy are in accord with the regions with larger MDV errors. The high consistency between the two free energy profiles not only confirms the convergence quality but also validates the quality of the collected samples, which we also used for the following structural analysis. As described earlier, the OST recursion should lead to faster convergence because its convergence is less dependent upon the sample size in the region where the OST recursion converges.

Briefly on the H_2O solute case, we performed a 73 ns OST simulation, during which six one-way trips were enabled. Within 10 ns, MDV dropped below 0.5 kcal/mol and within 30 ns, MDV dropped below 0.15 kcal/mol; and after 30 ns, three additional one-way trips occurred. Like the H_2S solute case, larger uncertainties are also distributed in the regions before H_2S enters the bilayer and when it diffuses around the membrane center.

Solute Trans-Membrane Free Energy Profiles

The TMFE surfaces of the two solutes are significantly different. Similar to what was reported previously^{8, 12}, the TMFE surface of the H_2O solute (Figure 2b) displays a simple bell shape with the free energy barrier located right in the middle of the bi-layer center. From the bulk solution to the membrane center, the free energy value monotonically increases and finally reaches 6.28 kcal/mol, which is very close to the water-to-hexadecane transfer free energy, 5.95 kcal/mol³⁰. In contrast, the TMFE surface of the H_2S solute (Figure 2a) has a more complex shape. At each side of the bi-layer, there is a free energy minimum around the interface between the polar head-group and non-polar acyl-chain regions; and at the membrane center, there is a free energy maximum. The free energy change between the bulk and the membrane center is about 0.12 kcal/mol, which is very close to the water-hexadecane transfer free energy, -0.02 kcal/mol ³⁰. Overall, the calculated bulk-to-membrane-center transfer free energies and the experimental water-to-hexadecane transfer free energies are in excellent agreement.

The transfer free energy values are summarized in Table 1. Comparing the transfer free energies of the two solute molecules, the difference is -6.16 kcal/mol from our OST results and -5.97 kcal/mol based on the experimental water-hexadecane partition coefficients³⁰. These values are consistent with the measured permeability ratio $P_{\text{H}_2\text{S}}/P_{\text{H}_2\text{O}}$ ^{4,5}, which was estimated to be $> 1.9 \times 10^4$ that corresponds to the free energy difference of -5.88 kcal/mol . Obviously, in consistence with the model by Walter and Gutknecht³¹, the transfer free energy difference of these solutes is the dominant factor that accounts for their large permeability difference.

Molecular Determinants of Trans-Membrane Free Energy Changes

What are the molecular determinants of the large transfer free energy difference between these solutes? To answer this question, we need to take a close look at how the molecules travel in the process, how they interact with various parts of the membrane, and particularly how structural changes correlate with the TMFE surfaces. For this purpose, we analyzed (a) the solute tilt angles to investigate solute orientation changes and (b) the oxygen atoms surrounding the solutes to understand how the solutes associate and dissociate with the polar parts of the environment. Specifically, based on Equation 4, we post-processed the OST simulation trajectories and calculated the normalized distributions ρ_z of the tilt angles (shown as $-RT \ln \rho_z$ in Figures 3a and 3b) and the numbers of surrounding oxygen atoms (shown as $-RT \ln p_z$ in Figures 4a and 4b) and further the average tilt angles (Figure 3c) and the average numbers of surrounding oxygen atoms (Figure 4c) at each membrane normal depth. Here, the tilt angle is defined as the angle between the molecular vector (from the O/S atom to the center of the hydrogen atoms) and the membrane normal vector; and as for surrounding oxygen atoms, we counted all of the oxygen atoms (including ones of both water molecules and lipid head groups) within 5 Å of the mass-weighted centers of the solutes.

Based on a common definition⁶, the POPC membrane environment can be divided to four distinct regions: the lipid tail end region (region 1: $-5\text{Å} < z < +5\text{Å}$); the lipid tail region (region 2: $+5\text{Å} < z < +12\text{Å}$ and $-12\text{Å} < z < -5\text{Å}$); the head-group region (region 3: $+12\text{Å} < z < +19\text{Å}$ and $-10\text{Å} < z < -12\text{Å}$); and the bulk solution region (region 4: $z > 19\text{Å}$ or $z < -1.9\text{Å}$). Correspondingly, the trans-membrane process can be separated to four sub-processes.

Diffusion from the bulk solution (region 4) to the membrane surface—Figure 3c shows the changes of the average tilt angles. In the bulk solution, there is no clear orientation preference. When approaching the membrane surface, the H₂O solute has its positively-charged hydrogen atoms slightly tilted towards the lipid head groups, whereas the H₂S solute displays a more complex orientation behavior: at the beginning, the sulfur atom is slightly rotated towards the membrane; around $z = \pm 2.7\text{Å}$, the molecule starts to spin in the opposition direction; and near the interface, the sulfur atom is re-oriented back towards the membrane surface. In this sub-process, the H₂O tilt angle always maintains a broad distribution (Figure 3b); as for H₂S, the closer to the membrane, the narrower the tilt angle distribution becomes and the distribution reaches its narrowest point at the bulk-bilayer interface (Figure 3a). Figure 4c shows the changes of the average numbers of surrounding oxygen atoms. In the bulk solution, both of the solutes are fully exposed to solvent; as is expected, the H₂O solute is better solvated. When approaching the membrane surface, their solvation levels slowly decrease. At the bulk-bilayer interface, the solvation level of the H₂O solute reaches a turning point at which the solvation level starts to decrease with a larger slope, while the corresponding turning point for H₂S occurs earlier (by 3Å). Before the solvation level reaches this turning point, the distribution of the number of surrounding oxygen atoms is fairly broad and largely constant.

During this sub process, the free energy change for H₂S is about −0.45 kcal/mol (Figure 2a), while the free energy of H₂O increases by 0.60 kcal/mol (Figure 2b). To the H₂O solute, this is a simple transition from a high dielectric bulk environment to a lower dielectric bulk-bilayer interface environment. Its tilt angle changes mainly reflect how, at different membrane normal positions, the progressively de-solvated H₂O dipole maximizes its interaction with the electrostatic field of the zwitterionic head-group region. As for the H₂S solute, when approaching the membrane surface, besides the unfavorable dipole de-solvation force, there apparently exists another type of force that favors the bulk-to-membrane diffusion. This reminds us about the fact that sulfur is indeed a hydrophobic moiety. To this portion of the free energy change (−0.45 kcal/mol), the favorable sulfur de-solvation contribution should be slightly larger, at least adequate to compensate the dipole de-solvation contribution. Because these two effects have distinct orientation preferences, it is very likely that the observed complex tilt angle changes may simply reveal how these two de-solvation forces competitively interplay at different membrane normal positions.

Diffusion across the head-group region (region 3)—Passing through the head-group region, the two solute molecules show almost identical behaviors in terms of the changes of the tilt angles and the numbers of surrounding oxygen atoms. In this sub-process, the solutes have their heavy atoms increasingly oriented towards the membrane interior and their hydrogen atoms increasingly rotated towards the bulk solution. The average tilt angle of H₂S has a larger change, decreasing from 80 to 45 degrees, while the average tilt angle of H₂O decreases from 98 to 72 degrees. At the same time, the numbers of surrounding oxygen atoms keep decreasing, from 6.7 to 3.2 for H₂O and from 5.4 to 1.6 for H₂S. Moreover, the distributions of the numbers of surrounding oxygen atoms are progressively narrower for both of the solutes.

When diffusing across the head-group region, despite seemingly similar molecular behaviors, the free energy changes of the two solute molecules are significantly different, with about 1.2 kcal/mol increases for H₂O and about 1.5 kcal/mol decrease for H₂S. The opposite free energy changes indicate that in this sub process, the governing forces for the two solute molecules are different. As is generally known, across the head-group region, the solvent water density decreases; correspondingly while region 3 is still largely polar, the dielectric constant progressively reduces. Therefore as for the polar H₂O solute, the polar-polar interaction with the environment should be increasingly weaker. H₂S has a much smaller dipole. Thus the free energy increase caused by the weakening of its polar-polar interaction with the environment should be quite modest. At the same time, the favorable sulfur de-solvation contribution should be dominant and progressively decrease before reaching the interface between the head group and acyl-chain regions, where the sulfur atom is fully excluded into the non-polar region of the bilayer. Therefore, although the two solutes display similar orientation behaviors, for H₂S, its average tilt angle decreases may be largely led by the favorable exclusion of the hydrophobic sulfur from the high dielectric environment; and for H₂O, the tilt angle decreases may be mainly led by the dipole de-solvation, which needs to be compensated by more persistent interactions between the positive end of the molecule with the electrostatic field of the zwitterionic head groups. The different causes for their tilt angle changes are supported by their different orientation

behaviors in the next sub process: the average tilt angle of H₂S reaches its minimum exactly at the interface between regions 2 and 3, whereas the average tile angle of H₂O continues to decrease when moving away from this interface.

Diffusion across the lipid tail region (region 3)—As discussed above, starting from the interface between regions 2 and 3, the average tilt angle change of H₂S immediately turns, while the average tilt angle of H₂O continues to decrease till reaching $z = \pm 7 \text{ \AA}$, where the average tilt angle starts to increase. In this sub process, the average numbers of surrounding oxygen atoms keep dropping. At the interface between regions 3 and 4, H₂S is completely de-solvated, while H₂O still maintains some level of solvation. Like in the earlier sub process, the distributions of the numbers of surrounding oxygen atoms also continue to be narrower.

Across region 3, the free energy of H₂S starts to increase and reaches its maximum value (0.12 kcal/mol) at the interface with region 4, whereas H₂O has a much large free energy increase (5.73 kcal/mol). These free energy changes simply reflect their progressive dissociation with the polar part of the bilayer environment.

From the above discussion, it is evident that while H₂O is a polar molecule, interestingly, its structural analogue, H₂S, is possibly one of the smallest amphipathic molecules, in which the sulfur moiety represents the hydrophobic end and its hydrogen atoms represent the hydrophilic end. Before the sulfur moiety fully diffuses into the non-polar acyl-chain region of the membrane, the sulfur de-solvation is the dominant effect that leads to the initial free energy decrease; and upon moving into the nonpolar region, the weakening of its polar interaction with the bilayer environment is the dominant effect that leads to the following free energy increase. Therefore, the amphipathic nature of H₂S is responsible for its favorable localization around the membrane interfacial region. To assess the sulfur de-solvation free energy, we compare the water-to-hexadecane transfer free energies of two pairs of sulfur-containing non-polar molecules including dimethyl sulfide (CH₃SCH₃)/dimethyl disulfide (CH₃SSCH₃) and diethyl sulfide (CH₃CH₂SCH₂CH₃) and diethyl disulfide (CH₃CH₂SSCH₂CH₃). For each pair, the experimentally measured transfer free energy difference (between the disulfide and the sulfide compounds) is similar with $-1.51 \text{ kcal/mol}^{30}$ for the pair of dimethyl compounds and $-1.31 \text{ kcal/mol}^{30}$ for the pair of diethyl compounds. As is generally known, solvation free energies are non-additive; particularly the de-solvation contribution of a hydrophobic specie in nonpolar molecule series tends to decrease with the increase of the molecular sizes. The membrane binding free energy of H₂S estimated by the OST simulation, -1.80 kcal/mol , not only qualitatively agrees with the experimental transfer free energy differences of two reference compound pairs, but also form a consistent trend with the transfer free energy differences of these reference compounds.

Concluding Remarks

From the recent investigations, hydrogen sulfide (H₂S) is identified as a new biologically important molecule. It is also found that this commonly known toxic gas compound possesses unique chemical features that allow it to quickly diffuse through cell membranes.

Employing the recent orthogonal space tempering (OST) method, we comparatively mapped the trans-membrane (POPC) free energy landscapes of H₂S and its structural analogue, water (H₂O). The calculated bulk-to-membrane-center transfer free energies are in excellent agreement with both the experimental water-to-hexadecane transfer free energies and the measured permeability ratio. Such agreement not only indicates that in accord with the Walter and Gutknecht model, the transfer free energy difference is the determining factor responsible for the large permeability gap between the H₂S and H₂O solutes but also confirms the sampling quality of the OST simulations.

Seeking to understand the molecular determinants for the transfer free energy difference between the two solutes, using the OST samples, we correlated the membrane normal dependent molecular orientations and numbers of surrounding oxygen atoms with the calculated TMFE surfaces. As shown, in contrast to the highly polar water solute, hydrogen sulfide is evidently amphipathic, and thus inside membrane is favorably localized at the interface between the polar head-group and non-polar acyl chain regions. Because the membrane binding affinity of H₂S is mainly governed by its small hydrophobic moiety; and the barrier height in between the interfacial region and the membrane center is largely determined by its moderate polarity, the trans-membrane free energy barriers to encounter are very small. Moreover when H₂S diffuses from the bulk solution to the membrane center, the above two effects nearly cancel each other, so as to lead to a negligible free energy difference. This study not only explains why H₂S can quickly pass through cell membranes but also provides a practical illustration on how to employ the OST free energy sampling method to conveniently analyze complex molecular processes.

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(a)

(b)

Figure 1.

The convergence behavior of the OST simulation on the H_2S trans-membrane permeation.

(a) The time-dependent changes of the membrane normal position. The magenta line: the interface between the bulk solution and the membrane bilayer; the red line: the interface between the head group and acyl-chain regions. (b) The time-dependent changes of the maximum deviation value. Inset: the map of the root mean square deviation (RMSD) between each pair of the free energy profiles.

Figure 2.

The trans-membrane free energy (TMFE) surfaces. (a) The TMFE surface of H₂S. The black curve: the TMFE surface directly from the OST recursion; the gray curve: the TMFE surface from the post-processing umbrella sampling re-weighting analysis. (b) The TMFE surface of H₂O. The uncertainties were obtained by the maximum deviation analysis.

Figure 3.

The tilt angles of the solute molecules at different membrane normal positions. (a) The normalized distributions ρ_z of the tilt angles of H₂S (shown as $-RT_o \ln \rho_z$). (b) The normalized distributions ρ_z of the tilt angles (shown as $-RT_o \ln \rho_z$) of H₂O. (c) The average tilt angles. The green curve: the average tilt angles of H₂S; the red curve: the average tilt angles of H₂O. The tilt angle is defined as the angle between the molecular vector (from the O/S atom to the center of the hydrogen atoms) and the membrane normal vector

Figure 4.

The numbers of surrounding oxygen atoms at different membrane normal positions. (a) The normalized distributions ρ_z of the number of oxygen atoms surrounding the solute H_2S (shown as $-RT_o \ln \rho_z$). (b) The normalized distributions ρ_z of the number of oxygen atoms surrounding the solute H_2O (shown as $-RT_o \ln \rho_z$). (c) The average numbers of surrounding oxygen atoms. The green curve: the average numbers of surrounding oxygen atoms; the red curve: the average numbers of surrounding oxygen atoms. The surrounding oxygen atoms are defined as oxygen atoms (including ones of both water molecules and lipid head groups) within 5 Å of the mass-weighted centers of the solutes.

Table 1

The summary of the transfer free energy values (in kcal/mol).

Solute	$G_{\text{Bulk} \rightarrow \text{Center}}(\text{calcd.})$	$G_{\text{water} \rightarrow \text{hexadecane}}(\text{exptl.})$	$G_{\text{calcd.} \rightarrow \text{exptl.}}$
H ₂ S	0.12	-0.02 ³⁰	0.14
H ₂ O	6.28	5.95 ³⁰	0.33

$G_{\text{H}_2\text{O} \rightarrow \text{H}_2\text{S}}(\text{calcd.})$	-6.16
$G_{\text{H}_2\text{O} \rightarrow \text{H}_2\text{S}}(\text{exptl.})$	-5.97
$-RT \ln \left(\frac{P_{\text{H}_2\text{S}}}{P_{\text{H}_2\text{O}}} \right)$	< -5.88 ^{4,5}

$G_{\text{Bulk} \rightarrow \text{Center}}(\text{calcd.})$: the calculated bulk-to-membrane-center transfer free energies; $G_{\text{water} \rightarrow \text{hexadecane}}(\text{exptl.})$: the experimentally measured water-to-hexadecane transfer free energies;