Supporting Information

Unassisted Transport of N-acetyl-L-tryptophanamide through Membrane: Experiment and Simulation of Kinetics

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Supporting Information Available:

1. Blocked tryptophan
2. Bilayer structure in the absence of permeant
3. Changes of the bilayer with permeant
4. Water penetration and hydrogen bonding

1. Blocked tryptophan

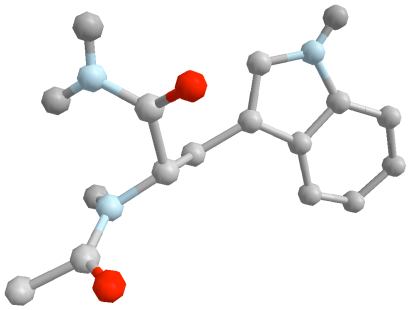

Figure S1: The molecular structure of the blocked tryptophan, N-acetyl-L-tryptophanamide (NATA) which is the permeant studied in the present manuscript. Oxygen atoms are red, nitrogens are blue and carbons and hydrogens are gray.

2. Bilayer structure in the absence of permeant

Figure S2 compares the total density profile of the DOPC lipid/water system (no permeant) with the experimental result from Liu and Nagle [1]. The membrane thickness as measured by the distance between the peaks is increased by about 3 Å with respect to the experimental result. We also observed a slight narrowing of the headgroup region and underestimation of the density of the hydrocarbon tail region with respect to the x-ray data. Siu et al. [2] compared structural properties of hydrated DOPC (a larger 128 lipid molecules system at 310 K) obtained with different force fields. Our results show peaks of the lipid density at 18 Å. Similar peaks were observed by them using the all-atom generalized AMBER force field. These peaks were less pronounced in their GROMACS simulations with the Berger force field. The separation between the phosphate peaks is

larger by at least 1 Å in our simulations compared to their GROMACS simulation. Our system was smaller and the simulations are conducted in the NVE ensemble (most other simulations are conducted in NPT with some constraining the surface tension as well). It is important to simulate microscopic kinetics using the NVE ensemble since this ensemble is free from phenomenological parameters such as friction coefficient and pressure coupling. It is well known that the friction coefficient influences the rate[3]. The differences in membrane structure with respect to the experiment are not large and encourage further calculations of permeation.

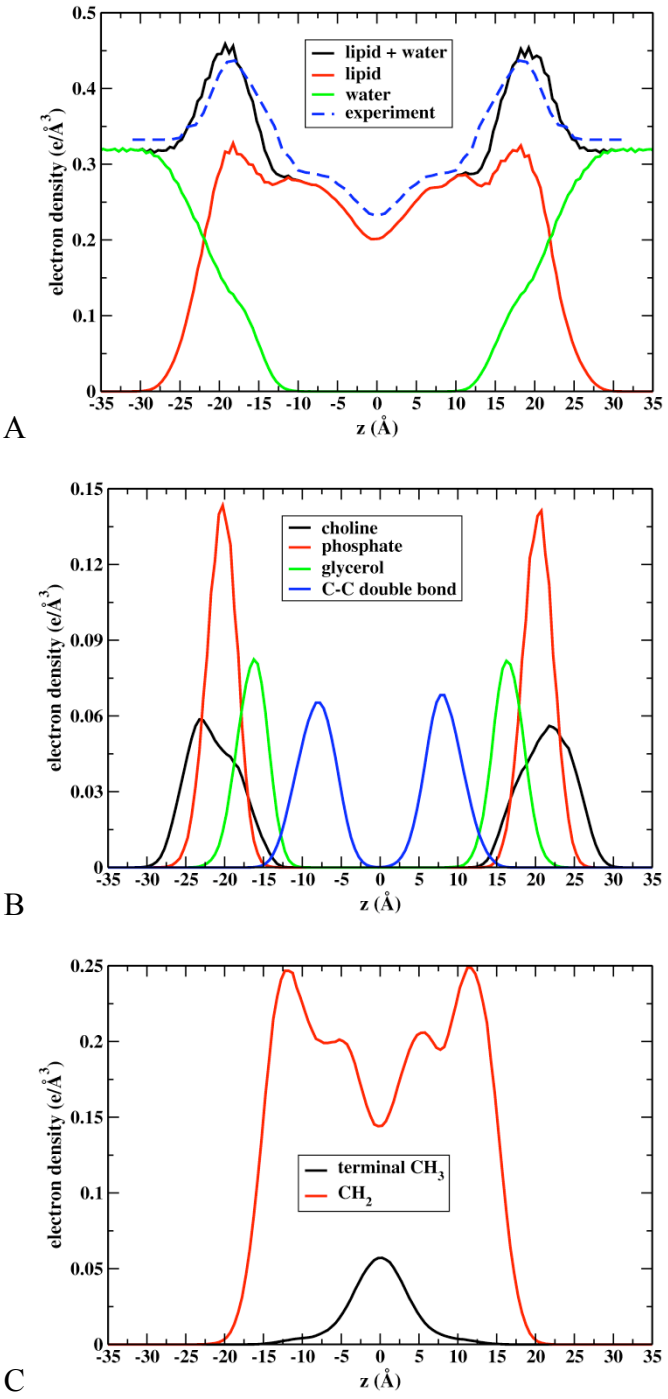

Figure S2: Electron density profiles of overall (A) and individual chemical groups (B and C) computed from the umbrella sampling simulations. In (A) we included the experimental profile from Liu and Nagle[60]. To facilitate comparison with experiment we

placed the appropriate number of electrons at the coordinates of each nucleus using bins of 0.5 Å along the bilayer normal and computed the electron density. The density profiles were computed independently for each layer in the bilayer. In the experiment, symmetry was used to depict the results for the second layer.

3. Changes of the bilayer structure with permeant

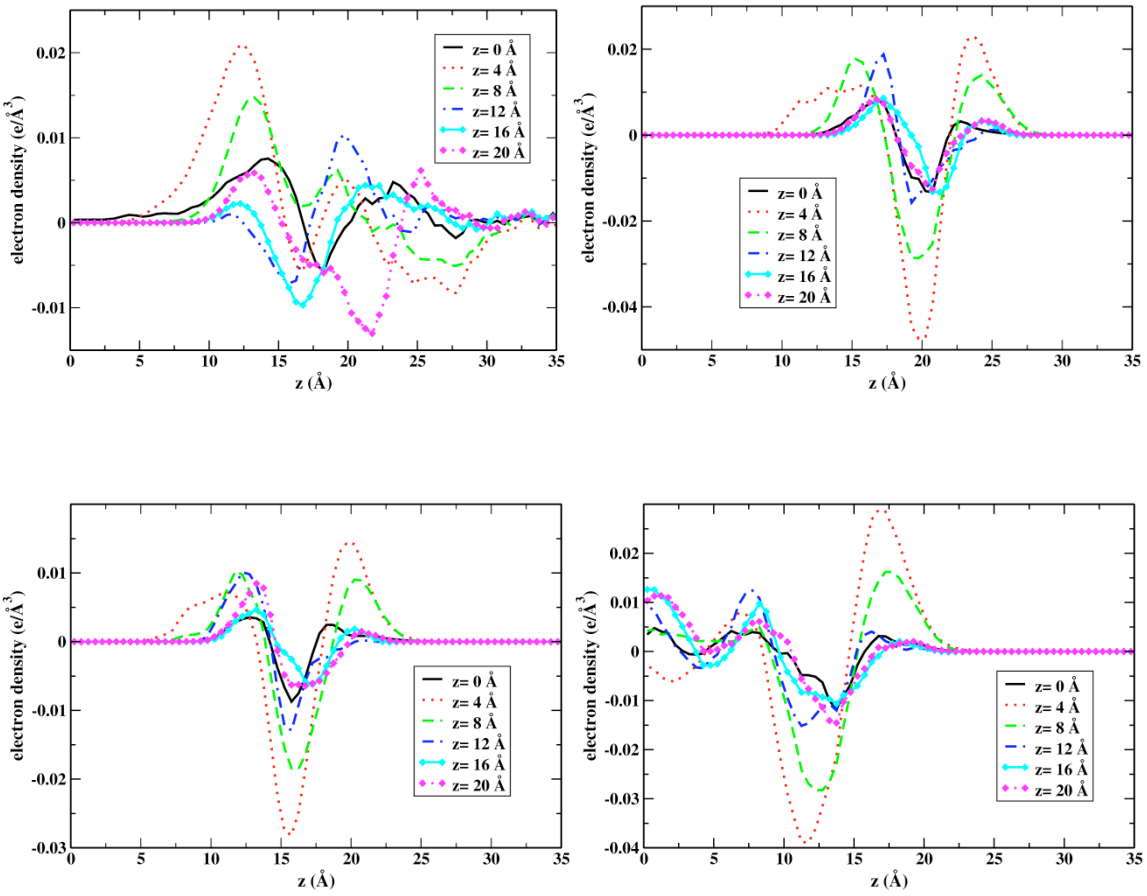

Figure S3: The difference between the electron density profiles with and without permeant are plotted along the z axis. The legends show the different depths of the permeant. The difference profiles are for water (top left), phosphate (top right), glycerol (bottom left) and CH_2 atoms (bottom right). These plots are average over the two layers

in the system with configurations taken from the last 20 ns of the corresponding umbrella sampling window.

4. Water penetration and hydrogen bonding

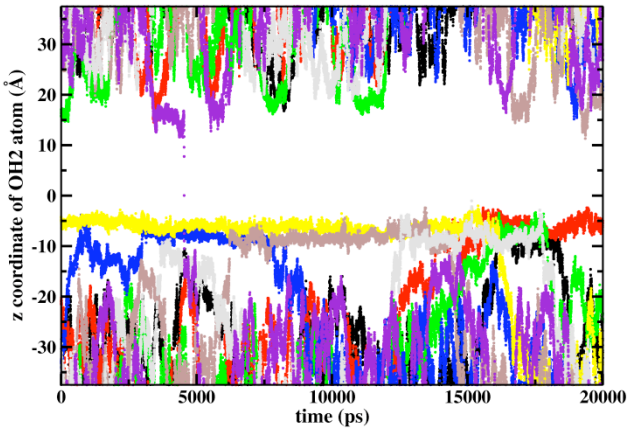

Figure S4: Position of the z coordinate of the oxygen atom of eight representative water molecules that come closer to the bilayer center. The center of mass of the permeant is at z = -3.5 Å. With the exception of the water molecule in purple all of the water molecules represented form hydrogen bonds with this NATA molecule. The figure shows the last 20 ns of a 50 ns constrained simulation. In that period of time, 57 different water molecules form hydrogen bond with the NATA molecule. The period of time a given water molecule is forming hydrogen bonds with the permeant vary. A typical long time is ~ 5 ns. But the figure shows that the longest live hydrogen bond can persist for more than 15 ns. During this simulation only one water molecule permeated completely (purple trace). This water molecule comes from the other side of the membrane and it did not form

hydrogen bonds with the permeant during its translocation. We did not observe water pemeation events in an independent simulation with the permeant at z = 3.5 Å.

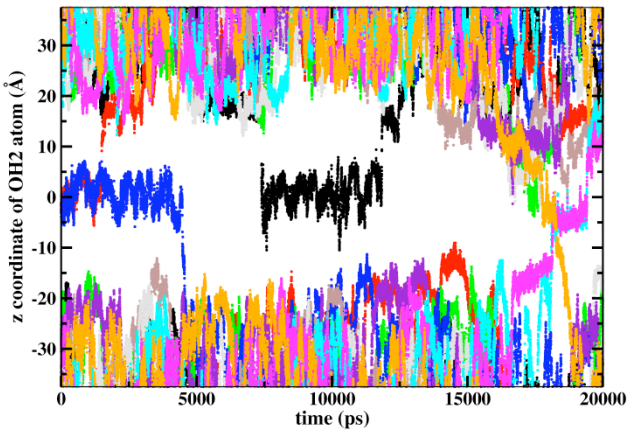

Figure S5: Position of the z coordinate of the oxygen atom of ten representative water molecules that come closer to the bilayer center. The center of mass of the permeant is at z = 0 Å. All of the represented water molecule form hydrogen bonds with that NATA molecule. The figure shows results from the last 20 ns of a 50 ns constrained simulation. In that period of time, 38 different water molecules form hydrogen bond with that NATA molecule. The time periods that water molecules stay closer to the permeant are shorter than in the previous figure. But three water translocations events are observed (orange, magenta and cyan trajectories). Two water permeation events were observed in a different independent simulation with the permeant at the same depth.

References

(1) Liu, Y. F.; Nagle, J. F. *Phys. Rev. E* **2004**, *69*.

(2) Siu, S. W. I.; Vacha, R.; Jungwirth, P.; Bockmann, R. A. *J. Chem. Phys.* **2008**, *128*.

(3) Hanggi, P.; Talkner, P.; Borkovec, M. *Rev. Mod. Phys.* **1990**, *62*, 251.