

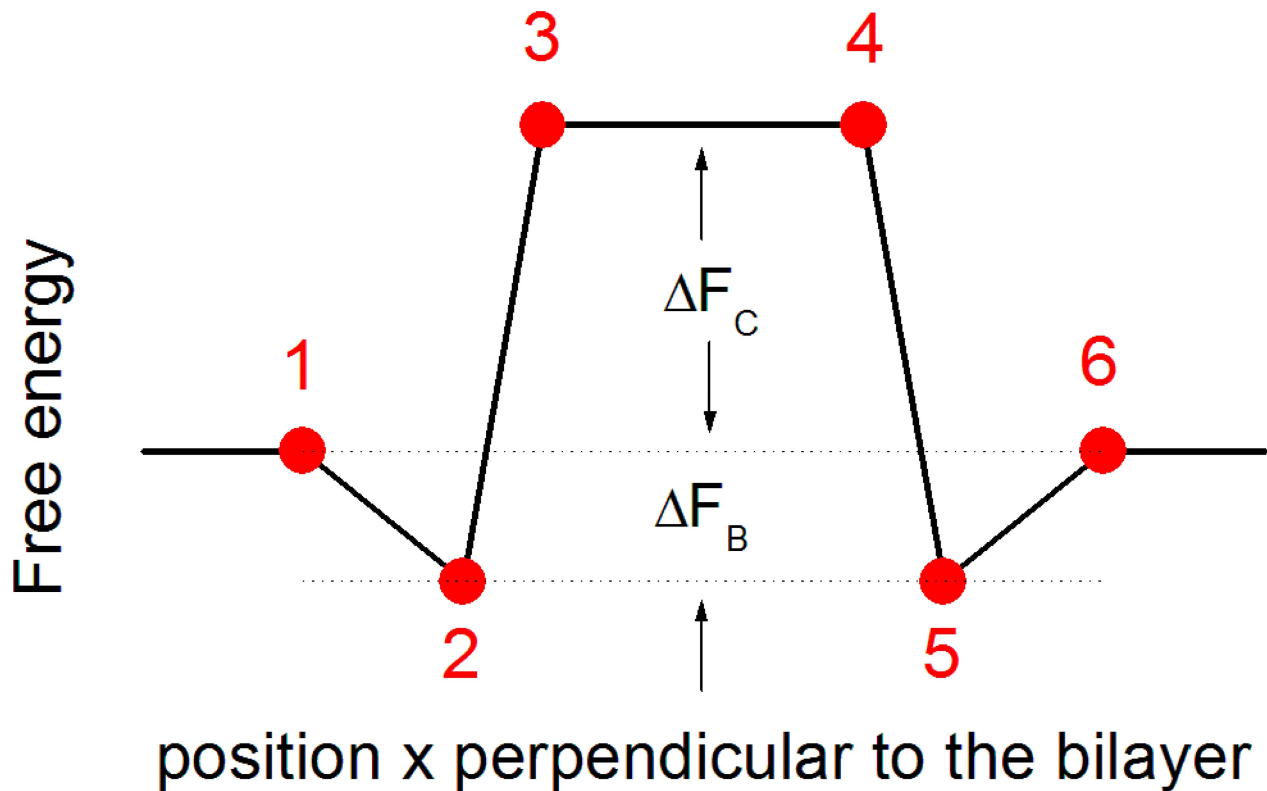
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The paper addressed the theory of permeability but the detailed analysis was limited to water as the solute. If the main idea is valid that there is structural factor  $(A - A_0)/A$  in the interfacial permeability  $P_H$  to account for the fraction of the total area  $A$  that is not blocked, then it should be possible to extend the theory to other solutes. However, as noted by Xiang and Anderson (2006) “a large body of evidence ... has shown that permeability is very sensitive to the chemical structure of the permeant.” In this supplemental material we consider the kinds of modifications in the mathematical models that should be made to consider other solutes. Of course,  $K$  will vary greatly with the solute. While it is customary to suppose that  $D_C$  may not vary much for small solutes,  $D_H$  could be different for different solutes depending upon the interaction of the solute with the complex interfacial headgroup region. Even  $A_0$  could change as the size of the solute changes, although both  $D_H$  and  $A_0$  should be the same for all lipids with the same headgroup. It should also be recognized that different solutes may have different free energy landscapes than the one shown in Fig. 4 as we shall see shortly.

Two classes of solutes will be considered in detail. The first is solutes, like acetic acid, that have been suggested to have strong binding to the interfacial region of bilayers (Xiang and Anderson, 1995). The second class is hydrophobic solutes whose partition coefficients into oil are greater than unity. The analyses will be performed for the simpler chemical kinetics model.

**A. SOLUTES BINDING TO THE MEMBRANE INTERFACE**

A solute with a hydrophobic end and a hydrophilic end may be expected to have its lowest free energy when it is situated with the obvious orientation at the hydrophobic/hydrophilic boundary of the membrane. This consideration leads to a free energy profile shown in Fig. S1. States 2 and 5 are the lowest free energy states in which the amphipathic solute is oriented at the interface. The partition coefficient in hydrocarbon is the usual  $K_C = \exp(-\beta\Delta F_C)$  but



**Figure S1.** The free energy landscape for acetic acid permeability. For the chemical kinetics model states 1 and 6 are at the bulk water boundaries, states 2 and 5 are the amphipathic binding sites, and states 3 and 4 are at the hydrocarbon core boundaries.

the overall partition coefficient into lipid bilayers is considerably larger (Xiang and Anderson, 1995) and this is accounted for in our model by the occupancy of low lying states 2 and 5.

The mathematical solution of the model in Fig. S1 is obtained in the same way as for the water model in Fig. 4. It may, however, be noted that this derivation will assume that the concentrations in all the states are small; this is valid for small enough concentrations of solute in water, but practical solute concentrations could saturate the interfacial binding sites 2 and 5 if the free energy difference  $\Delta F_B$  is large enough, and this would lead to nonlinear kinetics that will not be considered here.

It will be convenient to define  $K_B = \exp(-\beta\Delta F_B)$  and  $K_C = \exp(-\beta\Delta F_C)$  and to use the simplified notation,

$$\begin{aligned} k_C &= k_{34} = k_{43}, k_B = k_{32} = k_{45}, \\ K_B K_C k_B &= k_{23} = k_{54}, k_H = k_{12} = k_{65}, \quad (\text{S1}) \\ \text{and } K_B k_H &= k_{21} = k_{56}, \end{aligned}$$

Then the net forward currents between pairs of contiguous states are given by

$$J_{12} = d_H k_H (c_1 - K_B c_2) \text{ and } J_{56} = d_H k_H (K_B c_5 - c_6), \quad (\text{S2a})$$

$$J_{23} = d_B k_B (K_B K_C c_2 - c_3) \text{ and } J_{45} = d_B k_B (c_4 - K_B K_C c_5), \quad (\text{S2b})$$

$$J_{34} = d_C k_C (c_3 - c_4). \quad (\text{S2c})$$

In steady state, all  $J$ s are equal. Adding the two currents in each of Eqs. S2a and S2b, using Eq. S2c to eliminate  $c_3 - c_4$  in the sum of the currents in Eq. S2b and using that result to eliminate  $c_2 - c_5$  in the sum of the currents in the first line then gives  $J = P(c_1 - c_6)$ , with

$$1/P = (1/d_C k_C K_C) + (2/d_H k_H) + (2/d_B k_B K_C). \quad (\text{S3})$$

Identifying coefficients of diffusion as  $D_C = d_C^2 k_C$ ,  $D_B = d_B^2 k_B A / (A - A_0)$ , and  $D_H = d_H^2 k_H A / (A - A_0)$ , gives the permeabilities  $P_C$  of the hydrocarbon region,  $P_H$  of the head region, and  $P_B$  of the binding region as

$$\begin{aligned} P_C &= K_C D_C / d_C, P_H = (D_H / d_H) ((A - A_0) / A), \\ \text{and } P_B &= K_C (D_B / d_B) ((A - A_0) / A). \end{aligned} \quad (\text{S4})$$

Remarkably,  $K_B$  does not appear in these permeabilities. Because  $K_C$  is considerably smaller than unity,  $1/P_H$  is negligibly small. The common factor of  $K_C$  in  $P_C$  and  $P_B$  in Eq. S4 means that Overton's rule applies just as for the free energy landscape for water permeability in Fig. 4.

## B. HYDROPHOBIC SOLUTES

Let us consider a free energy landscape for a hydrophobic solute that has just four sites similar to the landscape in Fig. 4 except that the free energies of sites 2 and 3 are lower than the free energies of sites 1 and 4. Then, defining

$$\begin{aligned} K &= \exp(+\beta\Delta F), k_C = k_{23} = k_{32}, k_H = k_{12} = k_{43}, \\ \text{and } K^{-1} k_H &= k_{21} = k_{34}, \end{aligned} \quad (\text{S5})$$

one has the net forward currents between pairs of contiguous states

$$J_{12} = d_H k_H (c_1 - K^{-1} c_2) \quad (\text{S6a})$$

$$J_{23} = d_C k_C (c_2 - c_3) \quad (\text{S6b})$$

$$J_{34} = d_H k_H (K^{-1} c_3 - c_4), \quad (\text{S6c})$$

Equating the currents leads to

$$1/P = (1/d_C k_C K) + (2/d_H k_H). \quad (\text{S7})$$

Using the identification of the coefficients of diffusion, one then obtains

$$P_C = KD_C/d_C \text{ and } P_H = (D_H/d_H)((A - A_0)/A). \quad (\text{S8})$$

Again, the area-dependent factor in  $P_H$  corresponds to a partial obstruction of the flow through the headgroup region. However, unlike the results for hydrophilic solutes, Eq. S8 does not have a factor of  $K$  in  $P_H$ . Therefore, as solutes become more and more hydrophobic with increasingly larger values of  $K > 1$ , this predicts that the ratio  $P_C/P_H$  becomes even larger than for hydrophilic solutes. Thus, the rate-limiting step remains flow through the interfacial region, so a strong area dependence is predicted for hydrophobic solutes as for hydrophilic solutes. Eq. S8 also predicts that the double log plot of  $P$  with  $K$  would no longer be linear. It should be noted, however, that one should be concerned that a practical measurement of solute permeability when  $K \gg 1$  would require a large enough concentration of hydrophobic solute in water that the bilayer would be greatly perturbed by high concentrations of solute. A related consideration is nonlinear kinetics when the concentration of solute in the hydrocarbon region (i.e., sites 2 and 3 in the model) approaches saturation.

## REFERENCES

- Xiang, T.X., and B.D. Anderson. 1995. Phospholipid surface density determines the partitioning and permeability of acetic acid in DMPC:cholesterol bilayers. *J. Membr. Biol.* 148:157–167.
- Xiang, T.X., and B.D. Anderson. 2006. Conformational structure, dynamics, and solvation energies of small alanine peptides in water and carbon tetrachloride. *J. Pharm. Sci.* 95:1269–1287.