

Molecular Umbrella Transport: Exceptions to the Classic Size/Lipophilicity Rule

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This paper reports the consequences of molecular umbrella size and lipophilicity on their ability to transport a small polar agent (Cascade Blue) across lipid bilayers. In contrast to predictions based on classic solution-diffusion theory, transport rates were found to increase with increasing umbrella size (i.e., **1b** < **2b** < **3**, Chart 1). In addition, rate constants for the translocation step for **1a** and **1b** indicate that the less lipophilic umbrella (i.e., **1b**) crosses lipid bilayers more readily. The likely origin for this unusual umbrella behavior is briefly discussed.

One of the major goals in modern medicinal chemistry is to find ways of transporting polar drugs across hydrophobic barriers such as the plasma membrane of cells and the blood-brain barrier.^{1–7} To date, most approaches have relied on the “size/lipophilicity rule”, which originates from the solution-diffusion model for bilayer transport. According to this model, the permeability coefficient (*P*) of a permeant is directly proportional to its water–lipid partition coefficient (*K*) and its diffusion coefficient (*D*) and inversely proportional to the thickness (*x*) of the bilayer; that is, $P = (K \times D)/x$.^{1,6,7} Thus, an increase in lipophilicity (reflected by a higher *K*) or a decrease in the size of the permeant (reflected by a higher *D*) is expected to lead to increased permeability.

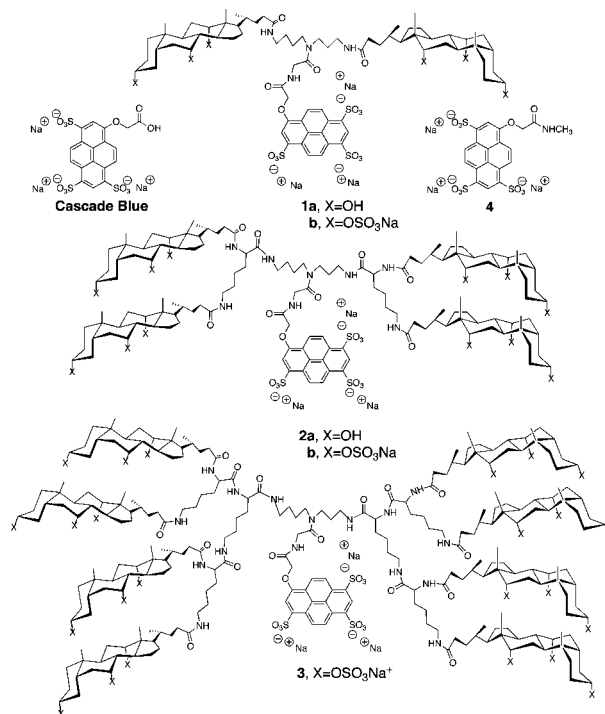
Our own approach to this problem has focused on the use of molecular umbrellas as transport vehicles.⁸ In essence, these molecules are composed of two or more facial amphiphiles (“walls”) attached to a central scaffold. A stylized illustration of a diwalled molecular umbrella is shown in Figure 1, where the shaded and unshaded rectangles represent a rigid lipophilic and hydrophilic face, respectively, and the oval represents the attached hydrophilic agent.

Our model for umbrella transport consists of initial adsorption to the membrane surface (A), followed by absorption into the interior of the membrane via the formation of a shielded conformation (B) (Figure 1). Subsequent translocation to the adjoining leaflet, 180° rotation, reversion to an adsorbed state, and release from the other side of the membrane then allows for net transport. Recent studies of **1a**, **1b**, **2a**, and **2b** indicate that A is their favored state when bound to lipid bilayers and that a subpopulation exists for the sulfated umbrellas, which lies deeper within the membrane.⁹

In principle, molecular umbrellas that have more walls could exhibit higher transport rates due to a greater shielding capacity. Alternatively, they could show lower transport rates due to a decreased diffusion coefficient. To distinguish between these two possibilities, and to probe the consequences of umbrella lipophilicity on membrane transport, we investigated the permeation behavior of a series of di-, tetra- and octawalled conjugates (**1a**, **1b**, **2a**, **2b**, and **3**) and a nonumbrella derivative of Cascade Blue (i.e., **4**). The method used for synthesizing the largest of these umbrellas, **3**, is described in the Supporting Information. Synthetic methods used to prepare **1a**, **1b**, **2a**, **2b**, and **4** have previously been reported.⁹

Binding measurements via equilibrium dialysis showed that the sulfated molecular umbrellas have very little affinity toward lipid bilayers but that the nonsulfated analogues are strongly bound. Thus,

Chart 1



using a 100 μ M umbrella solution and a 6.6 mM phospholipid dispersion, 85% of **1a** and 97% of **2a** became bound to the liposomes at 37 °C, while, under similar conditions, 1.21% of **1b**, 4.71% of **2b**, and 6.66% of **3** attached themselves to the liposomes. The former values correspond to partition coefficients, *K*, of 9.5×10^4 and 5.4×10^5 , while the latter correspond to 208, 835, and 1206, respectively.¹⁰

Liposomes (200 nm, extrusion) were prepared from 40 mg of a mixture of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylglycerol (POPG) [i.e., POPC/POPG, 95/5, mol/mol] and 2 mL of phosphate buffered saline [10 mM phosphate, 150 mM NaCl, pH 7.4, PBS], which was ca. 180 μ M in a given umbrella.^{8b} After dialysis at 23 °C for 48 h, to remove the umbrella from the external aqueous phase, the loss of umbrella that was still associated with the liposomes was monitored at 37 °C during extended dialysis.

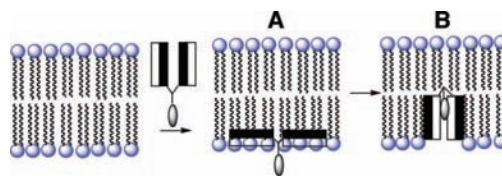


Figure 1. Stylized illustration of a diwalled molecular umbrella binding to a lipid bilayer in an exposed (A) and shielded (B) state.

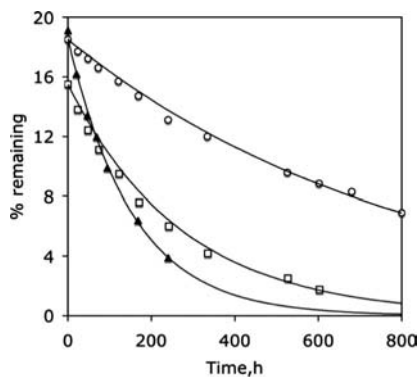


Figure 2. Plot of percent of **1b** (○), **2b** (□), and **3** (▲) that remains associated with liposomes made from POPC/POPG (95/5, mol/mol) as a function of dialysis time at 37 °C after an initial dialysis period of 48 h at 23 °C. Solid lines are first-order fits of the data.

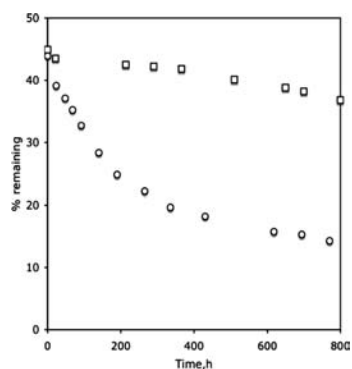
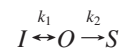


Figure 3. Plot of percent of **1a** (○) and **2a** (□) that remains associated with liposomes made from POPC/POPG (95/5, mol/mol) as a function of dialysis time at 37 °C after an initial dialysis period of 48 h at 23 °C.

Based on an average liposomal diameter of 200 nm (dynamic light scattering), the percent capture of the aqueous phase and umbrella is expected to be ca. 18%. As shown in Figure 2, capture of the weakly bound persulfated molecular umbrellas, **1b**, **2b**, and **3**, was in good agreement with this value. Subsequent efflux obeyed first-order kinetics with experimental rate constants, k_{exp} , of 0.0012, 0.0037, and 0.0065 h^{-1} for **1b**, **2b**, and **3**, respectively. Thus, observed transport rates increased with increasing umbrella size and were proportional to K .

These results are consistent with a steady state kinetic model in which the rate-determining step is the translocation of a weakly bound umbrella; that is, $k_{\text{exp}} = k_1 K L / (2W)$, where k_1 is the rate constant for translocation.¹⁰ For **1b**, **2b**, and **3**, this corresponds to k_1 values of 0.097, 0.075, and 0.091 h^{-1} . In the case of **4**, no significant release was observed after 300 h (not shown).

In sharp contrast, the release of strongly bound **1a** was biphasic in character while that of **2a** could barely be detected (Figure 3). The biphasic release of **1a**, together with a break point close to the “capture level”, indicates that the first stage is desorption of umbrellas bound to the exterior of the liposomes (O), and the slower second stage is the reversible translocation of umbrellas from the interior of the liposomes (I) to their exterior. These rates are consistent with the kinetic scheme



where S is the umbrella that is released to the external aqueous phase, $k_1 = 0.00047 \text{ h}^{-1}$ and $k_2 = 0.0060 \text{ h}^{-1}$ (see Supporting Information). In the case of **2a**, only the k_2 value can be calculated from the initial rate ($k_2 = 0.00037 \text{ h}^{-1}$). The much slower desorption of **2a** relative to **1a** reflects its stronger affinity to lipid bilayers.

The fact that the observed transport rates of **1b**, **2b**, and **3** are proportional to K indicates that membrane partitioning is a dominant factor. The similarity of their k_1 values further implies that a common rate-limiting step exists, which is most probably the detachment/partial dehydration of a “wet” fluorophore from the aqueous phase (Figure 1, structure **A**).¹¹ Because sulfated umbrella walls should be more highly hydrated than hydroxylated walls, detachment should be easier, which would account for the greater translocation rate (i.e., higher k_1 value) for the less lipophilic **1b** relative to **1a**.

The results presented in this paper highlight the fact that the crossing of lipid bilayers is a multistep process consisting of adsorption, dehydration, diffusion, rehydration, and desorption. Depending on the nature of the molecule to be transported, as reflected by the number, type, and distribution of polar groups, its shape, and its hydrophilic/lipophilic balance (HLB), any one of these steps can become rate-limiting. These considerations should weigh heavily in optimizing the design of drugs targeted to cross biological membranes.

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Supporting Information Available: Experimental procedures. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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- Binding data were analyzed using the nonsaturable partitioning model.⁷ If C_o is the equilibrium concentration of the conjugate in solution as measured in the absence of liposomes and C is the concentration in the presence of liposomes, then the partition coefficient is calculated as

$$K = \frac{(C_o - C)W}{C(L/2)}$$

Here, $L/2$ is the concentration of the lipids in the external half of the bilayer with a weighted average molecular weight of 649, and W is the concentration of water, 55.5 M.⁷

- For evidence against umbrella transport occurring through pores, see ref 8b.

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