

# Theoretical Analysis of Net Tracer Flux Due to Volume Circulation in a Membrane with Pores of Different Sizes

## *Relation to solute drag model*

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**ABSTRACT** When osmotic pressure across an artificial membrane, produced by a permeable electrically neutral solute on one side of it, is balanced by an external pressure difference so that there is no net volume flow across the membrane, it has been found that there will be a net flux of a second electrically neutral tracer solute, present at equal concentrations on either side of the membrane, in the direction that the "osmotic" solute diffuses. This has been ascribed to solute-solute interaction or drag between the tracer and the osmotic solutes. An alternative model, presented here, considers the membrane to have pores of different sizes. Under general assumptions, this "heteroporous" model will account for both the direction of net tracer flux and the observed linear dependence of unidirectional tracer fluxes on the concentration of the osmotic solute. The expressions for the fluxes of solutes and solvent are mathematically identical under the two models. An inequality is derived which must be valid if the solute interaction model and/or the heteroporous model can account for the data. If the inequality does not hold, then the heteroporous model alone cannot explain the data. It was found that the inequality holds for most published observations except when dextran is the osmotic solute.

### INTRODUCTION

When an osmotic pressure is generated across a frog skin or toad bladder by a permeable electrically neutral solute (such as sucrose or urea) placed on one side, a second electrically neutral tracer solute, at the same concentration on both sides will have a net flux across the membrane in the same direction as the flux of the first solute and in a direction opposite to net volume flow (Ussing, 1966; Franz and Van Bruggen, 1967; Biber and Curran, 1968; Ussing and Johansen, 1969). This phenomenon has been ascribed by Franz,

Galey, and Van Bruggen (1968) to solute-solute interaction (solute drag); i.e., the more concentrated "osmotic" solute drags the dilute "tracer" in the same direction as its diffusional flux. On the other hand, Ussing (1969) ascribed the phenomenon to a heterogeneous three-dimensional membrane structure which resulted in circulatory solvent flow within the membrane. The tracer, if confined to membrane regions where the direction of solvent flow is opposed to that of net volume flow, would be carried along in those regions by the solvent in a direction opposed to net volume flow. Ussing (1969) called this model "anomalous solvent drag."

The solute-solute interaction model postulates a large interaction term between the two solutes, as has been calculated by Franz, Galey, and Van Bruggen (1968) and Galey and Van Bruggen (1970) from observations on artificial membranes. These authors also applied an external pressure to the solution which contained the osmotic solute, the value of the pressure being chosen to yield zero net volume flow. Without the added pressure, tracer flowed in the same direction as volume flow. With it and at zero net volume flow, net tracer flux was in the same direction as the net flux of the osmotic solute. Unidirectional tracer fluxes varied linearly with the concentration of the osmotic solute, increasing with increasing concentration when the unidirectional flux was in the same direction as the osmotic solute flux, and decreasing with increasing osmotic solute concentration when it was in the direction opposite to osmotic solute flux. It should be noted that Ussing's explanation would not apply to the artificial membrane system, which would be expected not to have the geometry of his model.

While solute-solute interactions have been demonstrated in free solutions (cf. Curran, Taylor, and Solomon, 1967), and must operate to some extent in a porous membrane, in this paper we will consider an alternative model for tracer flows across an artificial membrane which emphasizes geometric factors. The membrane is assumed to be composed of pores of different sizes and is "heteroporous." Heteroporosity should produce volume circulation at zero net volume flow (Sollner, 1945; Rapoport, 1966). Some of the aspects of the irreversible thermodynamics of this model have been discussed previously by Kedem and Katchalsky (1963), and the model has been suggested also by Ussing and Johansen (1969). In treating it we will assume there is also solute-solute interaction. After the model has been analyzed, the solute-solute interaction model for a homogeneous membrane will be presented and will be shown to be mathematically identical to the model for a heteroporous membrane with or without solute-solute interaction. Therefore, the two models cannot be distinguished by experiments of the type heretofore performed, although an inequality involving only measurable quantities will be derived which must be satisfied if the heteroporous model without solute-solute interaction can by itself explain the data. Since solute-solute

interaction exists even in free solution and since it is most improbable that a membrane is completely homogeneous, both factors, solute-solute interaction and membrane heteroporosity, are present in any real membrane. However, their relative importance in accounting for experimental results cannot be determined at present.

## LIST OF SYMBOLS

$a$	"tracer" solute	$\sigma$	reflection coefficient
$b$	"osmotic" solute	$J_{v,i}$	volume flow through $i^{\text{th}}$ pore from left to right
$i$	$i^{\text{th}}$ pore	$J_v$	net volume flow through membrane from left to right = $\sum_i J_{v,i}$
$\bar{J}_a$	unidirectional flux of tracer from left to right	$c$	concentration in bathing solution
$\bar{J}_a$	unidirectional flux of tracer from right to left	$\Delta c$	difference in concentration of solute between right and left solutions
$J$	net flux from left to right	$\bar{c}$	an "average" concentration of the solute = $\Delta c / \ln [(c \text{ on right side}) / (c \text{ on left side})]$
$\omega$	permeability coefficient for heteroporous membrane model = $\sum \omega_i$	$L_{p,i}$	hydraulic conductivity of $i^{\text{th}}$ pore
$\omega^*$	permeability coefficient for homogeneous membrane model	$L_p$	hydraulic conductivity of homogeneous membrane model
$\omega_{ab}$	cross-permeability coefficient of tracer solute with osmotic solute for heteroporous membrane model = $\sum \omega_{ab,i}$	$P$	pressure difference across membrane, between right and left sides
$\omega_{ab}^*$	cross-permeability coefficient of tracer solute with osmotic solute for homogeneous membrane model	$R$	gas constant
		$T$	absolute temperature

## THEORY

The heteroporous membrane model is illustrated in Fig. 1. The membrane separates two well-stirred solutions. The electrically neutral osmotic solute is present in the right solution to which a pressure is applied so that net volume flow is zero. The membrane is composed of pores of different sizes, two of which are shown in the diagram.

A qualitative discussion of this model is as follows. Under the assumptions that there is no solute-solute interaction and that the reflection coefficient of the osmotic solute (cf. Kedem and Katchalsky, 1958) is smaller for the larger pore, there will then be a net volume flow from left to right through the smaller pore and an equal volume flow but in the opposite direction through the larger pore. For a given volume flow through any pore, the amount of material which can be "carried along" by that flow is larger the smaller the reflection coefficient of that material. If the reflection coefficient

for the tracer is smaller for the larger pore, then more tracer will be carried by the volume flow through the larger pore than through the smaller pore and the unidirectional tracer flux from right to left will be greater than in the opposite direction.

In order to quantify the heteroporous model, the linear equations and the approximations involved, as developed and discussed by Kedem and Katchalsky (1958), will be used.

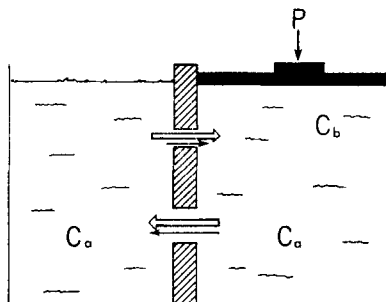


FIGURE 1. Heteroporous membrane model. Osmotic solute  $b$  is present at concentration  $c_b$  in the right solution, while tracer  $a$  is present at concentration  $c_a$  in both solutions. Solute-solute interaction is assumed not to be present. Pressure  $P$  is applied to the right solution so that the net volume flow,  $J_v$ , is zero. The membrane is represented as having a large and a small pore, with pore volume flows, represented by thick open arrows, equal in magnitude but opposite in direction. If the reflection coefficient of the tracer is assumed to be larger in the small pore, the unidirectional tracer flux (thin arrow) is greater in magnitude through the large than through the small pore. Therefore there is a net tracer flux from right to left.

For convenience, the “osmotic” solute will be assumed to be in the right solution and  $P$  will be the pressure difference between the right and left solutions.

The volume flow through the  $i^{\text{th}}$  pore, relative to the membrane, is given by

$$J_{vi} = L_{pi}(\sigma_{bi}RT\Delta c_b + \sigma_{ai}RT\Delta c_a - P). \quad (1)$$

Following the approach of Kedem and Katchalsky (1958), the flux equations of the electrically neutral osmotic and tracer solutes can be written as

$$J_b = -\omega_b RT\Delta c_b + \bar{c}_b \sum_i (1 - \sigma_{bi})J_{vi} - \omega_{ab}\bar{c}_b RT\Delta c_a \quad (2)$$

$$J_a = -\omega_a RT\Delta c_a + \bar{c}_a \sum_i (1 - \sigma_{ai})J_{vi} - \omega_{ab}\bar{c}_a RT\Delta c_b \quad (3)$$

where the  $\omega_{ab}$  terms represent solute-solute interaction contributions. Assuming that  $\sigma_{bi}$  is greater than or of the order of  $\sigma_{ai}$ , and since  $\Delta c_a \ll \Delta c_b$ , the

term involving  $\Delta c_a$  in equation (1) will be dropped, and since the osmotic solute is present only in the right solution,  $\Delta c_b = c_b$ . Thus, the net volume flow will be

$$J_v = \sum_i J_{vi} = \sum_i L_{pi}(\sigma_{bi}RTc_b - P), \quad (4)$$

and the unidirectional tracer fluxes become

$$\bar{J}_a = \omega_a RTc_a + \bar{c}_a \sum_i (1 - \sigma_{ai})J_{vi} - \omega_{ab}\bar{c}_a RTc_b, \quad (5)$$

$$\bar{J}_a = \omega_a RTc_a - \bar{c}_a \sum_i (1 - \sigma_{ai})J_{vi} + \omega_{ab}\bar{c}_a RTc_b, \quad (6)$$

where the tracer is present in the left solution for equation (5) and in the right solution for equation (6). If the tracer is present at equal concentrations on both sides of the membrane the net tracer flux would be

$$J_{a(\Delta c_a = 0)} = c_a \left[ \sum_i (1 - \sigma_{ai})J_{vi} - \omega_{ab}RTc_b \right]. \quad (7)$$

If the hydrostatic pressure balances the osmotic pressure, then  $J_v = 0$ . By solving for  $P$  from equation (4) and inserting this into equation (1), where the  $\Delta c_a$  term is neglected as discussed before, we have

$$J_{vi} = L_{pi}RTc_b \left( \sigma_{bi} - \frac{\sum_j L_{pj}\sigma_{bj}}{\sum_j L_{pj}} \right). \quad (8)$$

If  $\sigma_{bi}$  varies among pores, the individual  $J_{vi}$  will not all equal 0 and will vary in sign so that the volume circulation as illustrated in Fig. 1 will obtain. Substituting equation (8) into equations (5-7) yields,

$$\bar{J}_a = \omega_a RTc_a - RT\bar{c}_a c_b \left[ \omega_{ab} + \sum_i \sigma_{ai} L_{pi} \left( \sigma_{bi} - \frac{\sum_j L_{pj}\sigma_{bj}}{\sum_j L_{pj}} \right) \right], \quad (9)$$

$$\bar{J}_a = \omega_a RTc_a + RT\bar{c}_a c_b \left[ \omega_{ab} + \sum_i \sigma_{ai} L_{pi} \left( \sigma_{bi} - \frac{\sum_j L_{pj}\sigma_{bj}}{\sum_j L_{pj}} \right) \right], \quad (10)$$

$$J_{a(\Delta c_a = 0)} = -RT\bar{c}_a c_b \left[ \omega_{ab} + \sum_i \sigma_{ai} L_{pi} \left( \sigma_{bi} - \frac{\sum_j L_{pj}\sigma_{bj}}{\sum_j L_{pj}} \right) \right]. \quad (11)$$

Note that if the tracer is THO, then  $\sigma_{ai} = 0$  and if  $\omega_{ab} = 0$ , then  $J_a = 0$ , i.e. the unidirectional fluxes of THO would be equal, as has been found experimentally by Galey and Van Bruggen (1970). However, if  $\omega_{ab} \neq 0$ , this experimental result would not be expected. Further, if the parameters are independent of concentrations, the linear relations between unidirectional tracer fluxes and  $c_b$ , which are also found experimentally, are satisfied by equations (9) and (10).

We have not made any assumptions about the relative values of the  $\sigma_i$  in equation (11) but in the absence of electrostatic interactions or specific membrane-solute effects, it is reasonable to assume that a pore which has a smaller value of  $\sigma_i$  for solute  $a$  than another pore also will have a smaller value of  $\sigma_i$  for solute  $b$  than the other pore. Thus, the  $\sigma_i$  are assumed to be ordered for the pores and the following inequality will hold,

$$(\sigma_{ai} - \sigma_{aj})(\sigma_{bi} - \sigma_{bj}) > 0. \quad (12)$$

Equation (11) may be rearranged to yield

$$J_a = -RTc_a c_b \left[ \omega_{ab} + \sum_i \sum_j (\sigma_{ai} - \sigma_{aj})(\sigma_{bi} - \sigma_{bj}) L_{pi} L_{pj} / 2 \sum_k L_{pk} \right]. \quad (13)$$

Hence, if equation (12) is valid and  $\omega_{ab} \geq 0$ ,  $J_a < 0$ , which is the observation of Franz, Galey, and Van Bruggen (1968), and Galey and Van Bruggen (1970). That is, tracer will flow in the same direction as the flow of the osmotic solute when  $J_v = 0$  even if there were no solute-solute interaction.

We will now consider a "pure" solute-solute interaction model, in which the solute fluxes interact with each other in a homogeneous membrane. Analogously to equations (1)–(3), the flux equations for the homogeneous system can be written as

$$J_a = -\omega_a^* RT \Delta c_a + \bar{c}_a (1 - \sigma_a) J_v - \omega_{ab}^* \bar{c}_a RT \Delta c_b, \quad (14)$$

$$J_b = -\omega_b^* RT \Delta c_b + \bar{c}_b (1 - \sigma_b) J_v - \omega_{ab}^* \bar{c}_b RT \Delta c_a, \quad (15)$$

$$J_v = L_p (\sigma_b RT \Delta c_b + \sigma_a RT \Delta c_a - P). \quad (16)$$

For a given system, the independent variables are  $\Delta c_a$ ,  $\Delta c_b$ ,  $\bar{c}_a$ ,  $\bar{c}_b$ , and  $P$ , while the quantities that can be measured are  $J_a$ ,  $J_b$ , and  $J_v$ . These quantities can be used to calculate the coefficients  $\sigma_a$ ,  $\sigma_b$ ,  $L_p$ ,  $\omega_a^*$ ,  $\omega_b^*$ , and  $\omega_{ab}^*$  for the solute drag model and  $\omega_a$  and  $\omega_b$  for the heteroporous membrane model. The values of  $\sigma_{ai}$ ,  $\sigma_{bi}$ ,  $L_{pi}$ , and  $\omega_{ab}$  for the heteroporous membrane model cannot be determined from the measured quantities alone; they must be chosen so that sums of their various combinations satisfy the equations

and the experimentally measured quantities. If the coefficients of  $\Delta c_a$ ,  $\Delta c_b$ , and  $P$  are equated to each other for the two models (equations 1–4 for the heteroporous model and equations 14–16 for the solute drag model), a solution is found which places minimal constraints on the possible  $\sigma_i$ 's,  $L_{pi}$ 's, and  $\omega_{ab}$  which can be chosen. If these constraints can be satisfied, then the two models are mathematically identical and cannot be distinguished by experiments which measure the fluxes as concentration and pressure are varied. The constraints which are found are

$$\begin{aligned}
 \sigma_a &= \frac{\sum_i \sigma_{ai} L_{pi}}{\sum_i L_{pi}} \\
 \sigma_b &= \frac{\sum_i \sigma_{bi} L_{pi}}{\sum_i L_{pi}} \\
 L_p &= \sum_i L_{pi} \\
 \omega_a^* &= \omega_a + \bar{v}_a \left( \sum_i \sigma_{ai}^2 L_{pi} - \sigma_a^2 L_p \right) \\
 \omega_b^* &= \omega_b + \bar{v}_b \left( \sum_i \sigma_{bi}^2 L_{pi} - \sigma_b^2 L_p \right) \\
 \omega_{ab}^* &= \omega_{ab} + \sum_i \sigma_{ai} \sigma_{bi} L_{pi} - \sigma_a \sigma_b L_p.
 \end{aligned} \tag{17}$$

The first three relations in equation (17) are the same as those derived previously by Kedem and Katchalsky (1963) without the assumption of solute-solute interaction. The next two are also the same as those derived by Katchalsky and Kedem (1962) for the case of a membrane with two different pores and also without the assumption of solute-solute interaction. Since  $(\sum_i \sigma_i^2 L_{pi} / L_p - \sigma^2)$  is always positive, we see that in general  $\omega^* \geq \omega$ .

The final relation of equation (17) leads to an inequality among measurable quantities which can be used experimentally to distinguish between the models in certain cases. We will exclude the case in which solute transfer is more rapid than solvent transfer and  $\sigma$  is negative (cf. Kedem and Katchalsky, 1961), and will assume that the  $\sigma_i$ 's of the pores in the heteroporous model are positive and less than or equal to 1. If the heteroporous model is correct but the solute-solute interaction is negligible, i.e.  $\omega_{ab}$  may be taken equal to 0, and if the solute drag model is used to analyze the results, then if  $\sigma_a \leq \sigma_b$  (see Appendix I),

$$\omega_{ab}^* \leq \sigma_a(1 - \sigma_b)L_p, \quad (\sigma_a \leq \sigma_b) \tag{18}$$

while if  $\sigma_a \geq \sigma_b$

$$\omega_{ab}^* \leq \sigma_b(1 - \sigma_a)L_p \quad (\sigma_a \geq \sigma_b). \tag{19}$$

The maximum value of both equations (18) and (19) will occur when

$\sigma_a = \sigma_b = 0.5$ , so that a weaker inequality is

$$\omega_{ab}^* \leq L_p/4. \quad (20)$$

A conceivable experiment which might be used to distinguish between the models is the following. In the absence of applied pressure ( $P = 0$ ) and without a gradient of tracer across the membrane ( $\Delta c_a = 0$ ), volume flow across any pore will either be zero or from left to right if  $\sigma_{bi} \geq 0$ . The net flux of the tracer solute, under the heteroporous model with the assumption of negligible solute-solute interaction, will be from left to right and opposite to the direction of flux of the osmotic solute. Thus, if there is a net flux of the tracer at  $P = 0$  in the direction opposite to that of the volume flux, this would imply that the heteroporosity of the membrane alone is not sufficient to describe the system but that solute-solute interactions must also be considered.

If the parameters of the system are constant this experiment is equivalent to testing the inequalities found in equations (18) and (19). This may be seen as follows. Equation (14) for the case of  $P = \Delta c_a = 0$  reduces to

$$J_a = c_a(1 - \sigma_a)L_p(\sigma_b RT \bar{c}_b) - \omega_{ab}^* c_a RT \bar{c}_b. \quad (21)$$

If  $J_a \geq 0$ , i.e. the tracer moves in the direction opposite to that of the osmotic solute, then from equation (21) we see that  $\omega_{ab}^* \leq \sigma_b(1 - \sigma_a)L_p$ , which is the inequality of equation (19) but without the restriction that  $\sigma_a \geq \sigma_b$ . If  $\sigma_a \leq \sigma_b$ , then since

$$\sigma_b(1 - \sigma_a) \geq \sigma_a(1 - \sigma_b) \quad (\sigma_a \leq \sigma_b) \quad (22)$$

we see that the inequality of equation (18) is also satisfied for this case. Thus, the experiment at  $P = 0$  will not yield any further information than that which could be found from testing equations (18) and (19).

Another inequality on the  $\omega_{ab}^*$ , which may not be as experimentally useful, is derived in Appendix II. If  $\sigma_a + \sigma_b \leq 1$ , then if  $\omega_{ab} = 0$ ,

$$\omega_{ab}^* \geq -\sigma_a \sigma_b L_p, \quad (\sigma_a + \sigma_b \leq 1) \quad (23)$$

while if  $(\sigma_a + \sigma_b) \geq 1$ ,

$$\omega_{ab}^* \geq (\sigma_a + \sigma_b - 1 - \sigma_a \sigma_b)L_p \quad (\sigma_a + \sigma_b \geq 1). \quad (24)$$

If a third solute which is electrically neutral and impermeable is used at a low concentration, either in conjunction with or in place of an applied pressure, all the results which have been derived in this paper are still applicable. This is because whenever the term  $P$  was used previously, it can be replaced by  $(P - RT\Delta c)$  where  $c$  is the concentration of the impermeable solute.



## DISCUSSION

The inequalities of equations (18–20) were used to analyze the data of Galey and Van Bruggen (1970). If equation (14) of this paper is compared to equation (4) of their paper, we see that

$$\omega_{ab}^* = \frac{P_{12}}{c_a RT} \quad (25)$$

where the experimental values of  $P_{12}$  are listed in their paper. Since the value of  $c_a$  used in their experiment is 1 mM, and  $P_{12}$  is expressed in cm hr<sup>-1</sup>, equation (25) becomes

$$\omega_{ab}^* = 1.14 \times 10^{-8} P_{12} \quad (26)$$

where the units of  $\omega_{ab}^*$  are cm<sup>3</sup> dyne<sup>-1</sup> sec<sup>-3</sup>. The values of  $L_p$  and  $\sigma$  are listed in their Table I. A sample of the results of this calculation is shown in our Table I for the S & S B20 (Carl Schleicher and Schuell Company, Keene, N.H.) membrane for mannitol as the tracer.

TABLE I  
TEST OF RELATION  $\omega_{ab}^* \leq \sigma_a(1 - \sigma_b)L_p$  for  
S & S B20 MEMBRANE FOR MANNITOL AS TRACER  
(FROM GALEY AND VAN BRUGGEN, 1970)

$$L_p = 0.66 \times 10^{-10} \text{ cm}^3 \text{ dyne}^{-1} \text{ sec}^{-1}$$

$$\sigma_a = 0.055 \leq \sigma_b$$

Tracer (a)	Osmotic solute (b)	$\sigma_b$	$\omega_{ab}^*$	$\sigma_a(1 - \sigma_b)L_p$	$L_p/4$
Mannitol	Mannitol	0.055	0.014	0.034	0.165
	Sucrose	0.090	0.017	0.033	0.165
	Raffinose	0.10	0.012	0.033	0.165
	Dextran	$\leq 1$	0.30	$\approx 0$	0.165

Units of  $\omega_{ab}^*$ ,  $\sigma_a(1 - \sigma_b)L_p$ , and  $L_p/4$  are cm<sup>3</sup> dyne<sup>-1</sup> sec<sup>-1</sup>  $\times 10^{10}$ .

For the Diaflo UM-3 (Amicon Corporation, Cambridge, Mass.) and GA Type B (General Atomic Division of General Dynamics, San Diego, Calif.) membranes, the inequalities of equations (18) and (19) are satisfied for mannitol, sucrose, and raffinose by at least a factor of 5. For the S & S B20 membrane, the inequalities are also satisfied for mannitol, sucrose, and raffinose, but by a lesser factor which may be as low as 1.8. However,  $\omega_{ab}^* > L_p/4$  for the three solutes when dextran is the osmotic agent. This would imply that in order to explain the experimental results with dextran, solute-solute interaction must be considered and heteroporosity alone will not suffice. However, two points should be mentioned. The first is that when dextran is

used as the tracer, the  $\omega_{ab}^*$  is at least two orders of magnitude smaller than when dextran is the osmotic solute. In contrast to this, the  $\omega_{ab}^*$ 's are approximately equal for the tracer and osmotic solute conditions of mannitol, sucrose, and raffinose, respectively, even though the difference in concentration between tracer and osmotic solute for the latter three is much greater than for the dextran. Also, the concentration of dextran as an osmotic solute, approximately 160 g/1000 g H<sub>2</sub>O, is so high that the van't Hoff equation may not be valid (Charmasson, 1967). Both these factors raise serious questions as to the applicability of the linear equations of this paper to the experiment using dextran.

The experiments of Franz, Galey, and Van Bruggen (1968) with inulin and sucrose were not analyzed since the data were not compatible with the linear equations of this paper.

It should be emphasized that parameter agreement with the inequalities of equations (18) and (19) does not prove that the solute drag hypothesis is unimportant, but indicates only that the heteroporous membrane hypothesis is consistent with the data.

#### APPENDIX I

##### *Derivation of Equations (18) and (19)*

Let  $\sigma_a \leq \sigma_b$ . Since  $\omega_{ab} = 0$ , the relation for  $\omega_{ab}^*$  of equation (17) may be written as

$$\omega_{ab}^* = \left( \sum_i \sigma_{ai} \sigma_{bi} \frac{L_{pi}}{L_p} - \sigma_a \sigma_b \right) L_p. \quad (\text{A})$$

Since from equation (17)  $\sigma_a = \sum_i \sigma_{ai} L_{pi} / L_p$  and by assumption  $0 \leq \sigma_{bi} \leq 1$ , then

$$\sum_i \sigma_{ai} \sigma_{bi} L_{pi} / L_p < \sigma_a, \quad (\text{B})$$

and therefore

$$\omega_{ab}^* < \sigma_a (1 - \sigma_b) L_p. \quad (\text{C})$$

In order to derive equation (18), we must show that a case exists for which the inequality of equation (C) can be replaced by an equality. Consider a membrane in which  $\sigma_{ai}$  and  $\sigma_{bi}$  are either 0 or 1, and let  $\sigma_{bi}$  be 1 when  $\sigma_{ai}$  is 1. (Since  $\sigma_a \leq \sigma_b$ , this is possible.) In this case,  $\sum_i \sigma_{ai} \sigma_{bi} \frac{L_{pi}}{L_p} = \sigma_a$  and together with equation (C), equation (18) is proved.

An analogous argument can be made to derive equation (19).

## APPENDIX II

*Derivation of Equations (23) and (24)*

Since  $\sigma_{ai}$  and  $\sigma_{bi}$  are taken as nonnegative,  $\sum \sigma_{ai}\sigma_{bi}L_{pi} > 0$ . In order to derive equation (23), we must find a case for which this inequality can be replaced by an equality. First, let  $\sigma_a + \sigma_b \leq 1$  and let  $\sigma_{ai}$  and  $\sigma_{bi}$  be either 0 or 1, but when  $\sigma_{ai}$  is 1,  $\sigma_{bi}$  is 0 and therefore  $\sum_i \sigma_{ai}\sigma_{bi}L_{pi} = 0$ . Insertion into equation (17), remembering that  $\omega_{ab} = 0$ , yields equation (23).

Second, let  $\sigma_a + \sigma_b \geq 1$ , and define  $\sigma'_{ai}$  and  $\sigma'_{bi}$  such that  $\sigma'_{ai} = (1 - \sigma_{ai})$ ,  $\sigma'_{bi} = (1 - \sigma_{bi})$ . Further, define  $\sigma'_a$  and  $\sigma'_b$  such that

$$\begin{aligned}\sigma'_a &= \frac{\sum_i \sigma'_{ai}L_{pi}}{L_p} = 1 - \sigma_a \\ \sigma'_b &= \frac{\sum_i \sigma'_{bi}L_{pi}}{L_p} = 1 - \sigma_b.\end{aligned}\tag{D}$$

Therefore  $\sigma'_a + \sigma'_b \leq 1$  and by the argument used in the first case  $\sum_i \sigma'_{ai}\sigma'_{bi}L_{pi} \geq 0$ . Since

$$\begin{aligned}\sum_i \sigma_{ai}\sigma_{bi}L_{pi} &= \sum_i (1 - \sigma'_{ai})(1 - \sigma'_{bi})L_{pi} \\ &= L_p - \sigma'_a L_p - \sigma'_b L_p + \sum_i \sigma'_{ai}\sigma'_{bi}L_{pi}\end{aligned}\tag{E}$$

it follows that

$$\sum_i \sigma_{ai}\sigma_{bi}L_{pi} \geq L_p(1 - \sigma'_a - \sigma'_b) = L_p(\sigma_a + \sigma_b - 1).\tag{F}$$

Insertion of equation (F) into equation (17) with  $\omega_{ab} = 0$  yields equation (24).

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