Supplemental material

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1 Vesicle size dependence of the fusion rate constant

Due to the hydration repulsion water molecules attached to the charged membranes must be removed to overcome the energy barrier $\Delta E_{ij}$ of two fusing vesicles $i$ and $j$. For the shaded spherical caps ($\Delta O_i$ and $\Delta O_j$) in Fig. 9 which cover these molecules one must chose an appropriate parameter $\Delta$ defining the necessary interaction cross section to be considered. In the simplest case one may expect $\Delta E_{ij} \propto (\Delta O_i + \Delta O_j) = 2\pi r_i \Delta_i + 2\pi r_j \Delta_j$. For the two segments of $\Delta$, named $\Delta_i$ and $\Delta_j$ (see Fig. 9), a reasonable approximation is given by $\Delta_i = r_j/(r_i + r_j) \cdot \Delta$ and $\Delta_j = r_i/(r_i + r_j) \cdot \Delta$ which gives a compact form of the energy barrier entering Eq. (2):

$$\Delta E_{ij} = \frac{2\pi \Delta}{\lambda} \cdot \frac{r_i \cdot r_j}{r_i + r_j} = \frac{1}{\lambda_{\text{eff}}} \cdot \frac{r_i \cdot r_j}{r_i + r_j}, \text{ with } \lambda_{\text{eff}} = \lambda/(2\pi \Delta),$$

playing the role of an phenomenological proportionality factor.

2 Hyper-linear fusion rates

The total fusion rate $f_{ij}$ of two vesicles $i$ and $j$ is composed of individual fusion rates:

$$f_{ij} = f_{ij}(0) + f_{ij}(1) + ... + f_{ij}(n) = k_0 \cdot v_{ij}(0) + k_1 \cdot v_{ij}(1) + ... + k_n \cdot v_{ij}(n),$$

(1)

where $v_{ij}(m)$ describes the concentration of intermediates with $m$ FP pairs already formed (see Fig. 3A). We assume rapid equilibria between successive intermediates $v_{ij}(m)$ and $v_{ij}(m+1)$:

$$v_{ij}(m + 1) = \frac{(n - m)(n - m)}{m + 1} \cdot K^* v_{ij}(m),$$

where the factor $(n-m)(n-m)$ describes the combinatorial multiplicity of pairing options of one additional FP pair and $(m+1)$ accounts for the possible unpairing processes. It can be easily shown that

$$v_{ij}(m) = \binom{n}{m} \binom{n}{m} \cdot m! \cdot (K^*)^m \cdot v_{ij}(0),$$

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with \( v_{ij}(0) = K \cdot v_i v_j \). Eq. (1) can then be rewritten in a compact form:

\[
f_{ij} = K \cdot v_i v_j \sum_{m=0}^{n} k_m \binom{n}{m} \binom{m}{l} \cdot (K^*)^m.
\]

We assume that a successful fusion can be accomplished primarily if at least \( n \) FP pairings could be established (overcome of energy barrier), i.e. \( k_0,...,k_{n-1} \ll k_n \). The accelerating effect of SNARE pairing is accounted for by the association constant \( K^* \) describing the stabilisation of the fusion intermediate by SNARE pairing. If no pre-formation of aggregates is assumed \( K^* \) will certainly depend linearly on the concentration of total available FPs: \( K^* \propto A_i A_j \);

\[
f_{ij} = K \cdot v_i v_j \cdot k_n \cdot n! \cdot A_i^a A_j^b = \kappa' \cdot A_i^a A_j^b \cdot v_i v_j,
\]

with \( \kappa' = K \cdot k_n \cdot n! \). In the case of two independent FPs A and B, one retrieves

\[
f_{ij} = \kappa' \cdot (A_i^a + B_i^a, B_j^b) \cdot v_i v_j,
\]

where \( \kappa' \) accounts for the vesicle size dependent association constant \( K \) (repulsive hydration force). A specific assumption of this dependency yields Eq. (5).

In the scenario of pre-forming aggregates (see Fig. 3B) a concerted transition from \( v_{ij}(0) \) to \( v_{ij}(n) \) is assumed to proceed by a single step. In this case \( K^* \) depends on the number of aggregates instead of single FPs giving rise to a Hill type cooperativity as described in Eq. (6).

3 De novo generation of unit size vesicles

The enrichment of unit vesicles with either A or B type FPs results in coatA or coatB unit vesicles, respectively. For the initial de novo generation of such vesicles as well as for the budding process from organelles (see next section) we need a quantifier to describe the degree of biased loading during these two processes. We therefore introduce an enrichment factor \( \eta > 1 \) entering the two generation processes. As a consequence \( \alpha_A^a \) and \( \alpha_B^a \) are not two simple rates but rather two (symmetrical) binomial distributions with respect to \( a \):

\[
\alpha_A^a = \alpha_0 \binom{Z}{a} \cdot \frac{\eta}{\eta + 1}^a \cdot \frac{1}{\eta + 1}^{Z-a}
\]

\[
\alpha_B^a = \alpha_0 \binom{Z}{a} \cdot \frac{1}{\eta + 1}^a \cdot \frac{\eta}{\eta + 1}^{Z-a},
\]

with \( a = 0,...,Z \) describing the \( Z+1 \) possible numbers of loaded A type FPs. It has to be noted that if the number of A type FPs in a unit size vesicle is given, the number of B type FPs is determined as \( b = Z - a \), where \( Z \) describes the total number of FPs in a unit size vesicle.

4 Budding matrices \( \{A_i^{-1} \gamma_{a_i} \} \) and \( \{B_i^{-1} \gamma_{a_i} \} \)

The parameter \( \eta > 1 \) describes the increased loading affinity of an A type FP to its corresponding vesicle compared to the affinity of a B type FP to such a vesicle. Symmetrically, \( \eta \) characterises the preferential loading of coatB-based vesicles with B type FPs. As an example the \( \{A_i^{-1} \gamma_{a_i} \} \) matrix entries are calculated in the following. The probabilities \( P_A \) and \( P_B \) of loading a single A or B type FP, respectively, to a coatA vesicle which buds off from an organelle of size \( i \) with \( a_i \) FPs are given as:

\[
P_A(a_i, b_i) = \frac{\eta \cdot a_i}{\eta \cdot a_i + b_i}
\]

\[
P_B(a_i, b_i) = \frac{b_i}{\eta \cdot a_i + b_i}.
\]
These elementary probabilities enter the recursion formula for $A_{\gamma a_i}(m, n)$ describing the successive loading of a vesicle with at present $m$ FPs comprising $n \leq m$ FPs of type A:

$$A_{\gamma a_i}(m, n) = A_{\gamma a_i}(m - 1, n - 1) \cdot P_A(a_i - n + 1, iZ - a_i + n - m) + A_{\gamma a_i}(m - 1, n) \cdot P_B(a_i - n, iZ - a_i + n + 1 - m),$$

for $m = 1, ..., Z$, $n = 0, ..., (a_i - a_i - 1)$ and $A_{\gamma a_i}(0, n) = 0 \forall n \neq 0$, $A_{\gamma a_i}(0, 0) = 1$. The budding matrices in Eq. (7) are finally determined by

$$A_{\gamma a_i}^{a_i - 1} = A_{\gamma a_i}(Z, a_i - a_i - 1).$$