

Pathways for fast and slow fusion of nanovesicles without membrane rupture: Electronic Supplementary Information

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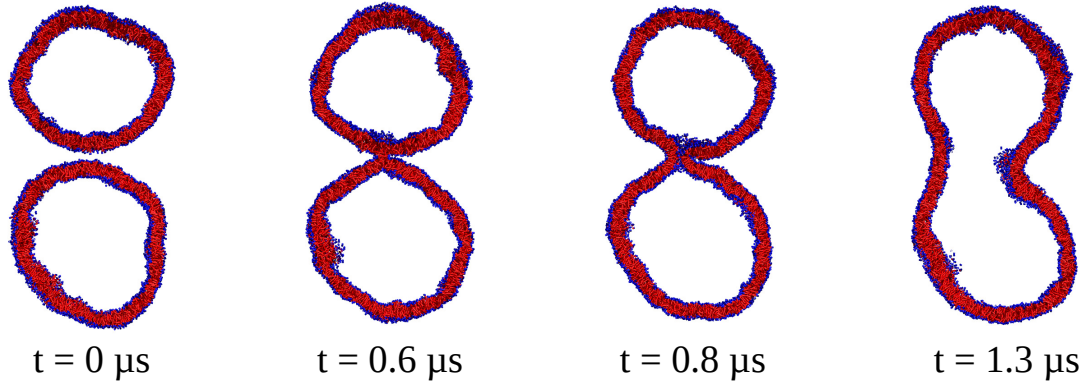
- S1 Outcome of fusion simulations for different values of the lipid number N_{ol} in the outer leaflet, the lipid number $N_{il} = 10100 - N_{ol}$ in the inner leaflet, and the dimensionless volume parameter $\nu = \nu_0$ corresponding to tensionless vesicle bilayers with leaflet tensions Σ_{ol} and Σ_{in} S1

Supplementary Table S1: Outcome of fusion simulations for different values of the lipid number N_{ol} in the outer leaflet, the lipid number $N_{il} = 10100 - N_{ol}$ in the inner leaflet, and the dimensionless volume parameter $\nu = \nu_0$ corresponding to tensionless vesicle bilayers with leaflet tensions Σ_{ol} and Σ_{in} in units of $k_B T/d^2$. The numerical error of the two leaflet tensions is of the order of $\pm 0.05 k_B T/d^2$. For each parameter set, $n_{traj} = 9$ statistically independent simulations are studied, each with a run time of t_{traj} in μs . These simulations lead to n_{fast} fast fusion events and n_{slow} slow fusion events with $n_{traj} = n_{fast} + n_{slow}$. The last column displays the average fusion time $\langle t_f \rangle$ in μs as obtained by averaging over the fusion times of all n_{traj} trajectories.

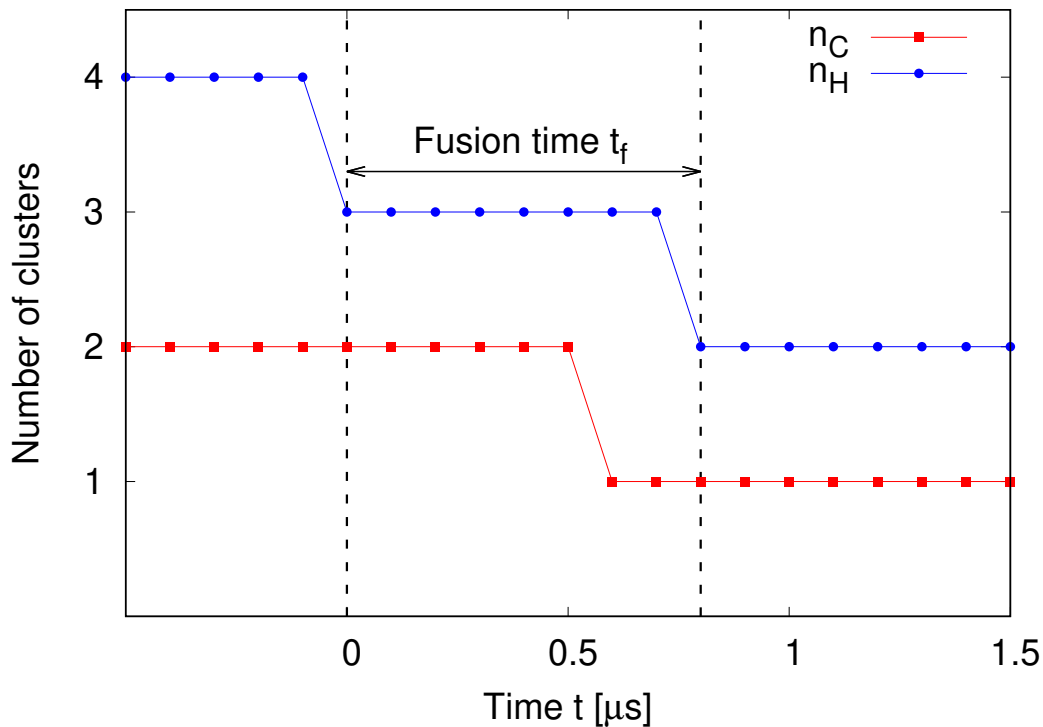
N_{ol}	N_{il}	$\nu = \nu_0$	Σ_{ol}	Σ_{il}	n_{traj}	t_{traj}	n_{fast}	n_{slow}	$\langle t_f \rangle [\mu s]$
5500	4600	0.966	1.36	- 1.35	9	15	9	0	1.4
5600	4500	0.966	1.11	- 1.09	9	15	8	1	3.1
5700	4400	0.966	0.86	- 0.82	9	30	1	8	15.8
5900	4200	0.967	0.28	- 0.25	9	> 140	0	0	> 140

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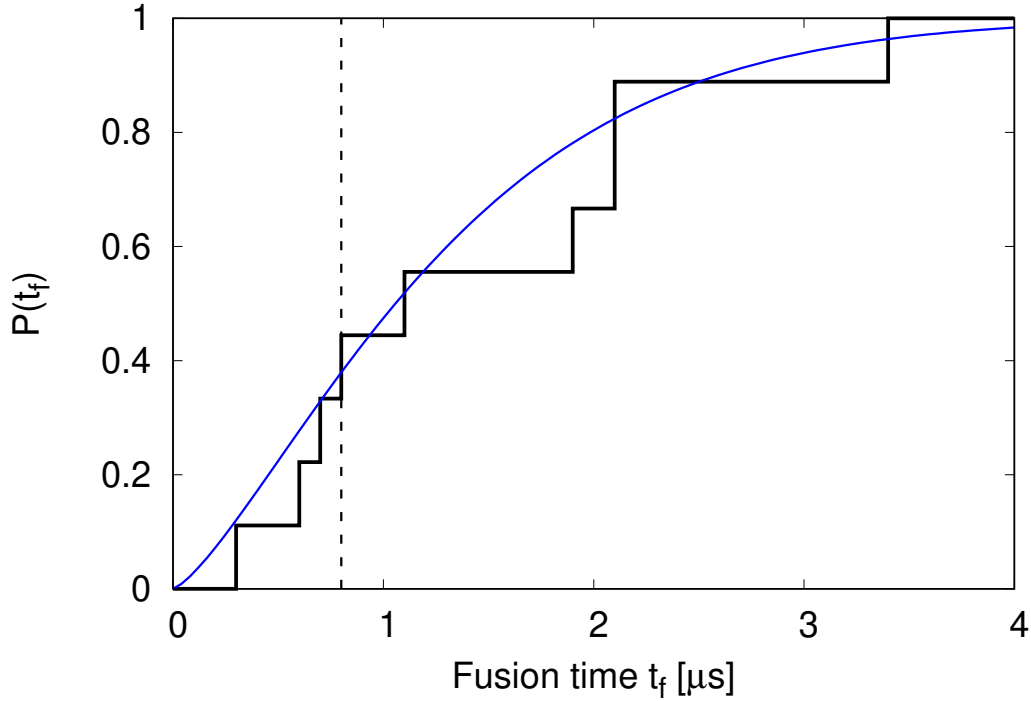
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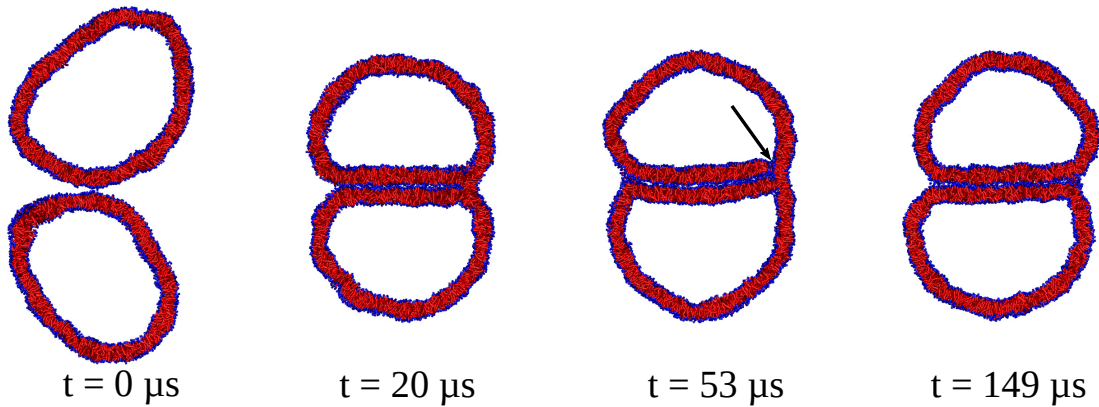
Supplementary Figure S1: Snapshots from a simulation trajectory in which two identical vesicles with $N_{\text{ol}} = 5500$, $N_{\text{il}} = 4600$ and $\nu = 0.966$ undergo fast fusion. Lipid tails are shown in red and lipid head-groups in blue. Time $t = 0 \mu\text{s}$ is defined as the moment at which the two vesicles get in contact. A hemifusion stalk is formed at $t = 0.6 \mu\text{s}$. The vesicles fuse at $t = 0.8 \mu\text{s}$. The fusion occurs before the vesicles could flatten as a result of their adhesion.



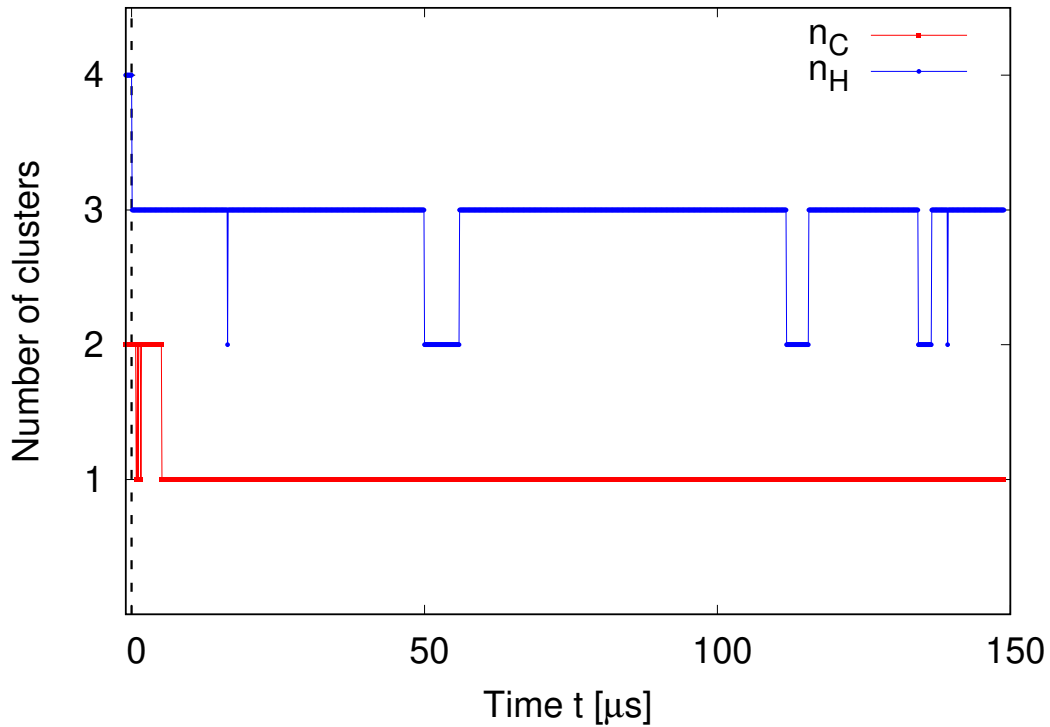
Supplementary Figure S2: Cluster analysis of a simulation trajectory in which two identical vesicles with $N_{\text{ol}} = 5500$, $N_{\text{il}} = 4600$ and $\nu = 0.966$ undergo fast fusion. The number n_H of clusters of lipid headgroups is shown in blue. The number n_C of clusters of lipid chains is shown in red. At $t < 0$, $n_H = 4$ and $n_C = 2$, because that the two vesicles make no direct contact. At $t = 0$, the n_H value drops from 4 to 3 while $n_C = 2$, which reflects the first contact of the the outer leaflets of the two vesicles. At $t = 0.6 \mu\text{s}$, the n_C value drops from 2 to 1 while $n_H = 3$, which results from hemifusion stalk formation. At $t = 0.8 \mu\text{s}$, the n_H value drops from 3 to 2 while $n_C = 1$, which reflects fusion neck formation. Thus, the fusion time $t_f = 0.8 \mu\text{s}$ in this case, as indicated by the horizontal arrow. The corresponding snapshots are shown in Fig. S1.



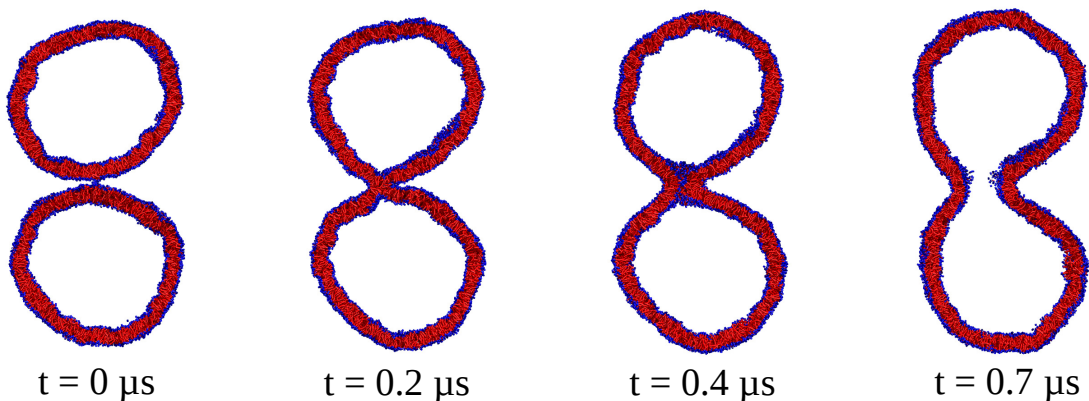
Supplementary Figure S3: Cumulative probability distribution for the fusion time of two identical vesicles with $N_{ol} = 5500$, $N_{il} = 4600$, and $\nu = 0.966$. The blue line represents the Weibull distribution, $P(t_f) = 1 - \exp(-(t_f/\tau)^k)$, where τ and k are fitting parameters. Here, $\tau = 1.4 \mu\text{s}$ and $k = 1.3$. The average fusion time is $\langle t_f \rangle = 1.4 \mu\text{s}$. The vertical dashed line indicates $t_f = 0.8 \mu\text{s}$ and corresponds to the fusion process shown in Figs. S1 and S2.



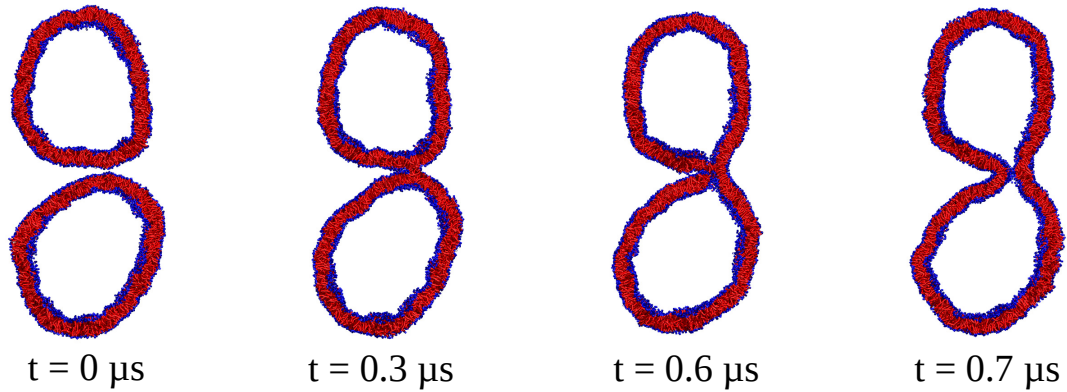
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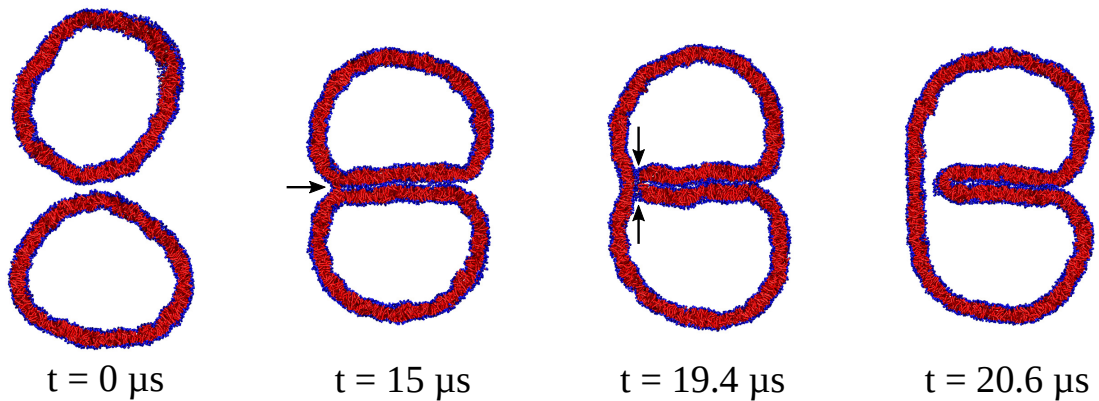
Supplementary Figure S5: Cluster analysis of a simulation trajectory of two identical vesicles with $N_{ol} = 5900$, $N_{il} = 4200$ and $\nu = 0.967$. The number n_H of clusters of lipid headgroups is shown in blue. The number n_C of clusters of lipid chains is shown in red. The two vesicles come into contact at $t = 0 \mu s$, which results in a change of the headgroup cluster number from $n_H = 4$ to $n_H = 3$ while $n_C = 2$. The subsequent adhesion state displays some recurrent changes of the chain cluster number between $n_C = 2$ and $n_C = 1$ until it is replaced by a hemifused state with $n_H = 3$ and $n_C = 1$ at $t = 4.5 \mu s$. Furthermore, as shown in Fig. S4 for the time point $t = 53 \mu s$, the hemifused state exhibits transient pores, the opening and closure of which change the headgroup cluster number n_H between $n_H = 3$ and $n_H = 2$. The corresponding snapshots are shown in Fig. S4.



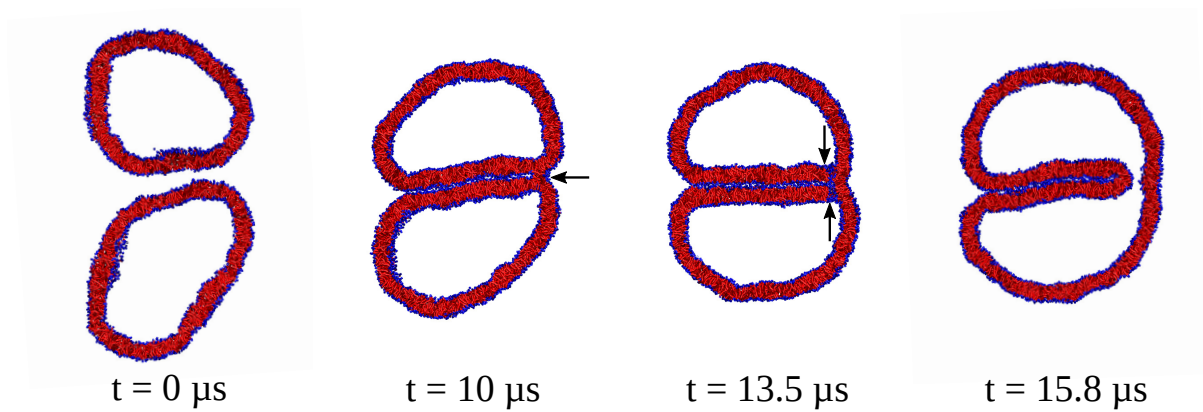
Supplementary Figure S6: Snapshots from a simulation trajectory in which two identical vesicles with $N_{ol} = 5500$, $N_{il} = 4600$ and $\nu = 1$ undergo fast fusion. Lipid tails are shown in red and lipid head-groups in blue. Time $t = 0 \mu s$ is defined as the moment at which the two vesicles get in contact. At $t = 0.2 \mu s$ a hemifusion stalk is formed. At $t = 0.4 \mu s$ a pore through both of the vesicle bilayers can be observed. The vesicles fuse at $t = 0.7 \mu s$. The fusion occurs before the vesicles could flatten as a result of their adhesion. The fusion time $t_f = 0.7 \mu s$ in this trajectory.



Supplementary Figure S7: Snapshots from a simulation trajectory in which two identical vesicles with $N_{\text{ol}} = 5600$, $N_{\text{il}} = 4500$ and $\nu = 1$ undergo fast fusion. Lipid tails are shown in red and lipid head-groups in blue. Time $t = 0$ is defined as the moment at which the two vesicles get in contact. At $t = 0.3 \mu\text{s}$ a hemifusion stalk can be observed. At $t = 0.6 \mu\text{s}$ a pore through the vesicle bilayers can be observed. The vesicles fuse at $t = 0.7 \mu\text{s}$. The fusion occurs before the vesicles could flatten as a result of their adhesion. The fusion time $t_f = 0.7 \mu\text{s}$ in this trajectory.



Supplementary Figure S8: Snapshots from a simulation trajectory in which two identical vesicles with $N_{\text{ol}} = 5700$, $N_{\text{il}} = 4400$ and $\nu = 1$ undergo slow fusion. Lipid tails are shown in red and lipid head-groups in blue. Time $t = 0$ is defined as the moment at which the two vesicles get in contact. A hemifusion stalk can be observed in the snapshot at $t = 15 \mu\text{s}$ in a spot on the rim of the adhesion area, as indicated by the horizontal arrow. At $t = 19.4 \mu\text{s}$, a pore through both of the vesicle bilayers can be seen, as indicated by the vertical arrows. The fusion occurs at $t = t_f = 20.6 \mu\text{s}$, i.e. long after the vesicles formed an extended contact area as a result of their adhesion.



Supplementary Figure S9: Snapshots from a simulation trajectory in which two identical vesicles with $N_{\text{ol}} = 5700$, $N_{\text{il}} = 4400$ and $\nu = 0.9$ undergo slow fusion. Lipid tails are shown in red and lipid head-groups in blue. Time $t = 0$ is defined as the moment at which the two vesicles get in contact. A hemifusion stalk can be observed in the snapshot at $t = 10 \mu\text{s}$. The hemifusion stalk is located in a spot on the rim of the adhesion area, as indicated by the horizontal arrow. At $t = 13.5 \mu\text{s}$, a pore through both of the vesicle bilayers is formed. This pore is indicated by the vertical arrows. The fusion occurs at $t = t_f = 15.6 \mu\text{s}$, i.e. long after the vesicles formed an extended contact area as a result of their adhesion.