Supplementary Information

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1 Two-body potential

Here we reproduce the definition of the two body potential $V_B^{xy}(r)$ between a linker x and a linker y shown in [1,2]:

$$V_B^{xy}(r) = \frac{4\lambda_x\lambda_y\lambda_m \,\mathrm{kei}(q_0 r)}{\pi^2(\lambda_m^2+\lambda_x\lambda_y+\lambda_x\lambda_m+\lambda_y\lambda_m) - 16\lambda_x\lambda_y\,\mathrm{kei}(q_0 r)} \left(\pi\Delta l_x\Delta l_y + \left(\frac{2\Delta l_x^2\lambda_x}{\lambda_x+\lambda_m} + \frac{2\Delta l_y^2\lambda_y}{\lambda_y+\lambda_m}\right)\mathrm{kei}(q_0 r)\right) + \frac{1}{2}\log\left(1 - \frac{16\lambda_x\lambda_y\,\mathrm{kei}(q_0 r)^2}{\pi^2(\lambda_x+\lambda_m)(\lambda_y+\lambda_m)}\right). \quad (1)$$

Here, $\Delta l_i = h_0 - l_i$, q_0 describes the inverse of the correlation length, and λ_m is the effective spring stiffness of the membrane, related to the inverse of the fluctuation amplitude of the unbound membrane[3].

When assuming a tensionless membrane of stiffness κ, residing in a minimum of a potential with a curvature γ, $q_0 = 1/\sqrt[4]{\kappa/\gamma}$ and, $\lambda_m = 8\sqrt{\kappa\gamma}$. Analogous equations can exist for membranes with tension[3].

In the simulations, the particles are prevented from residing at the same lattice site, which in the potential has the effect of a hard-core repulsion below a distance given by the lattice spacing a. Therefore we modify the potential as follows

$$V_2^{xy}(r) = \begin{cases} \infty & r \le a \\ V_B^{xy}(r) & r > a. \end{cases} \quad (2)$$

2 Expected coordination number

In the random system containing N_1 links of type 1 and $A-1-(N_1-1) = A-N_1$ lattice sites that are empty or of type 2, the average probability to find a type 1 bond on any one lattice site is

$$\frac{N_1-1}{A-1}, \quad (3)$$

and therefore the average number of neighbours is given by

$$n_b^e = \frac{4(N_1-1)}{A-1}. \quad (4)$$

Another way to see this is by considering the average number of neighbours by the probability of finding n neighbours ($p(n)$) on the available 1 to 4 neighbouring lattice sites $\sum_{i=1}^{4} n\cdot p(n)$, which can be calculated by the probability of drawing either a type 1 bond or not from the total number of bonds,

$$n_b^e = 1\cdot\frac{\binom{N_1-1}{1}\binom{A-N_1}{3}}{\binom{A-1}{4}} + 2\cdot\frac{\binom{N_1-1}{2}\binom{A-N_1}{2}}{\binom{A-1}{4}} + 3\cdot\frac{\binom{N_1-1}{3}\binom{A-N_1}{1}}{\binom{A-1}{4}} + 4\cdot\frac{\binom{N_1-1}{4}\binom{A-N_1}{0}}{\binom{A-1}{4}} = \frac{4(N_1-1)}{A-1}. \quad (5)$$

3 Monte Carlo Simulations

Benefitting from a recently developed coarse-grained Monte Carlo simulation framework[2,4], we conduct Monte Carlo simulations on the growth of adhesion domains with multiple binding pairs to mimic the current experiments as closely as possible. Specifically, the GUV and SLB membranes are discretized into square lattices with lattice constant a. Periodic boundary conditions are considered here. A growing contact zone with an increasing radius is located in the middle of the simulation box, while the DNA linkers on the free region between GUV and SLB membranes maintain constant density, providing the reservoir reconstructing the appropriate statistical ensemble. The simulation system evolves in time by alternate execution of diffusion, stochastic binding/unbinding as well as growth steps. The mobility of freely diffusive DNA linkers is treated as a random walk with time step Δt. Here, D_{DNA} represents the diffusion coefficient of DNA constructs which is taken to be the same for all DNA lengths because they all have the same membrane attachments. The forming and breaking of intermittent bridges between the two membranes follow a coarse-grained kinetics[5]. DNA linkers can switch from an intra- to an inter-membrane configuration only if the opposing lattice site is empty, while breaking the bridge places the intra-membrane DNA construct on the SLB or the vesicle with equal probability. The effective association/dissociation rates coupling to the thermodynamic properties of membranes can be analytically obtained. In addition, the contact zone radius will increase at a slow

constant rate, 0.001 per time step, from initially zero to the final state with $R = 30$, so that the concentration of bridges is always in equilibrium with the instantaneous area of contact between the two membranes. The further details of our Monte Carlo simulations are described in Ref.[2,4]. Using the representative system parameters summarized in Table 1 of the manuscript, we can perform a series of simulations and understand the DNA linker mediated membrane adhesion.

4 Membrane parameters

The full list of parameters used in the simulation is shown in Table 1. The simulation box is made from N cells with lattice constant a. The contact zone grows at a constant rate $\Delta R/\Delta t$ that ΔR is the incremental radius of contact zone at each time step, Δt. The diffusion coefficients of both short and long free DNA constructs are represented by D_{DNA}. The immobilization of DNA bridges is considered. C_S^0 and C_L^0 stand for the initial concentrations of short and long DNA constructs, respectively. $C_{\mathrm{B}S}$ and $C_{\mathrm{B}L}$ are the initial concentrations of short and long DNA bridges. The interaction between single chol and its binding site in the bilayer is associated with the insertion energy E_{i}, interaction range α_S, α_L and intrinsic reaction rate k_{0S}, k_{0L}. Due to common design of the sticky ends, E_{i} is identical for the short and long linker. The remaining parameters are explained in the table caption in the main text.

Table 1 Full list of membrane parameters used in the simulation.

Quantity	Symbol	Value
Lattice constant	a	10 nm
Number of cells	N	80×80
Growth rate of the contact zone	$\Delta R/\Delta t$	0.05 nm
Diffusion coefficient of L_{S} and L_{L}	D_{DNA}	$0.16\,\mu\mathrm{m}^2/\mathrm{s}$
Diffusion coefficient of bridges		0
Initial concentration of DNA	$C_S^0+C_L^0$	50%
Initial concentration of bridges	$C_{\mathrm{B}S}$, $C_{\mathrm{B}L}$	0
Time step	$\Delta t = a^2/4D_{\mathrm{DNA}}$	$156.2\,\mu$s
Membrane bending rigidity	κ	$30\,k_{\mathrm{B}}T$
Potential curvature	γ	$2.17 \times 10^5\,k_{\mathrm{B}}T/\mu\mathrm{m}^4$ [6,7]
Potential height	h_0	50 nm [6,7]
Temperature	T	300 K
Fluctuations amplitude	σ_0	7 nm [6,7]
DNA linker elastic modulus	λ_S, λ_L	$3 \times 10^4\,k_{\mathrm{B}}T/\mu\mathrm{m}^2$ [8]
DNA linker length	l_S	8 nm [8]
	l_L	15 nm [8]
Insertion energy	E_{i}	$6\,k_{\mathrm{B}}T$ [9]
Interaction range	$\alpha_S=\alpha_L$	1 nm [4]
Intrinsic reaction rate	$k_{0S}=k_{0L}$	$1000/\mathrm{s}$ [10]

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