Electronic Supplementary Material (ESI) for Soft Matter.

Supplementary Information:

Interplay between cooperativity of intercellular receptor-ligand binding and coalescence of nanoscale lipid clusters in adhering membranes

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This document consists of two sections. The first one conains a detailed description of the mean-field calculation. The second section contains eight figures that supplement the main results.

Mean field theory

We implemented the MF theory for adhering membranes without bending rigidity contrast between the raft patches and membrane matrix, i.e., $\kappa_{\rm r}^{\rm o} = \kappa_{\rm m}^{\rm o}$. We started with the *grand-canonical* Hamiltonian

$$\mathcal{H} = \mathcal{H}_{\rm ad} - \mu_{\rm r} \sum_i \left(n_i^+ + n_i^-\right) - \mu_{\rm p} \sum_i \left(m_i^+ + m_i^-\right) \tag{S1}$$

where $\mu_{\rm r}$ and $\mu_{\rm p}$ are the chemical potentials of the patches occupied by rafts and proteins, respectively. Transforming membrane composition variables $n_i^{\rm o} = 0, 1$ to spin variables $s_i^{\rm o} = 2n_i^{\rm o} - 1 = \pm 1$, and using MF approximation $s_i^{\rm o} s_j^{\rm o} \approx \langle s_i^{\rm o}\rangle s_i^{\rm o} + \langle s_j^{\rm o}\rangle s_j^{\rm o} - \langle s_i^{\rm o}\rangle\langle s_j^{\rm o}\rangle$ with the average $\langle s_i^{\rm o}\rangle = \langle s_j^{\rm o}\rangle$, we obtained MF Hamiltonian

$$\begin{aligned} \mathcal{H}_{\rm MF} &= \mathcal{H}_{\rm me} + \mathcal{H}_{\rm R-L} + \\ &\quad \sum_{\rm o=+,-} \sum_i \left[\varepsilon - U_{\rm eff} s_i^{\rm o} - \frac{U_{\rm a}}{2} s_i^{\rm o} m_i^{\rm o} - \left(\frac{U_{\rm a}}{2} + \mu_{\rm p}\right) m_i^{\rm o}\right] \end{aligned} \tag{S2}$$

with $\varepsilon = \frac{1}{2}\left(Us^2 - U - \mu_{\rm r}\right)$ and $U_{\rm eff} = Us + U + \frac{1}{2}\mu_{\rm r}$, where $s = \langle s_i^+\rangle = \langle s_i^-\rangle$ because of the up-down symmetry of membrane system. Here, the membrane bending energy $\mathcal{H}_{\rm me}$ reduces to $\mathcal{H}_{\rm me} = \sum_i \kappa_{\rm eff}/(2a^2)(\Delta_{\rm d} l_i)^2$ with the effective bending rigidity $\kappa_{\rm eff} = \kappa_{\rm m}^+\kappa_{\rm m}^-/(\kappa_{\rm m}^+ + \kappa_{\rm m}^-)$. The grand-canonical partition function that sums over all configurations of the two membranes specified by the separation field $\{l_i\}$ and over all degrees of freedom of both raft and protein patches takes the form

$$\begin{aligned} \mathcal{Z}_{\rm MF} &= \left[\prod_i \int_0^\infty {\rm d}l_i\right] \left[\prod_i \sum_{s_i^+=\pm 1} \sum_{s_i^-=\pm 1} \sum_{m_i^+=0,1} \sum_{m_i^-=0,1}\right] e^{-\beta \mathcal{H}_{\rm MF}} \\ &= e^{-2N\beta\varepsilon} \left[\prod_i \int_0^\infty {\rm d}l_i\right] \left[e^{-\beta\mathcal{H}_{\rm me}} \prod_i \sum_{\sigma^+=\pm 1} \sum_{\sigma^-=\pm 1} w_{\sigma^+,\sigma^-}(l_i)\right] \end{aligned} \tag{S3}$$

with $\beta = (k_{\rm B}T)^{-1}$ and

$$\begin{aligned} w_{\sigma^+,\sigma^-}(l_i) &= \Big[1 + e^{\beta\left(\frac{\sigma^+ + 1}{2} U_{\rm a} + \mu_{\rm p}\right)} + e^{\beta\left(\frac{\sigma^- + 1}{2} U_{\rm a} + \mu_{\rm p}\right)} \\ &\quad + e^{\beta\left(\frac{\sigma^+ + \sigma^-}{2} U_{\rm a} + U_{\rm a} + 2\mu_{\rm p}\right)} e^{\beta U_{\rm b}\theta(l_{\rm b}/2 - |l_i - l_{\rm c}|)}\Big] \cdot \\ &\quad e^{\beta U_{\rm eff}(\sigma^+ + \sigma^-)} \end{aligned} \tag{S4}$$

By defining $A_{\sigma^+,\sigma^-} = w_{\sigma^+,\sigma^-}(l_i)|_{\theta(l_b/2-|l_i-l_c|)=0}$ and $B_{\sigma^+,\sigma^-} = w_{\sigma^+,\sigma^-}(l_i)|_{\theta(l_b/2-|l_i-l_c|)=1}$, the partition function given by Eq. (S3) can be rewritten as

$$\begin{aligned} \mathcal{Z}_{\rm MF} &= e^{-2N\beta\varepsilon}\Big[\sum_{\sigma^+=\pm1}\sum_{\sigma^-=\pm1} A_{\sigma^+,\sigma^-}\Big]^N \cdot \\ &\quad \Big[\prod_i \int_0^\infty {\rm d}l_i\Big] e^{-\beta\left[\mathcal{H}_{\rm me}+\sum_i V_{\rm b,eff}(l_i)\right]} \end{aligned} \tag{S5}$$

where the effective binding potential $V_{\rm b,eff}(l_i) = -U_{\rm b,eff}\theta(l_b/2-|l_i-l_c|)$ is a square-well potential of the same width l_b and location l_c as the R-L binding potential in Eq. (2) in the main text. The effective binding strength

$$U_{\rm b,eff} = k_{\rm B}T \ln \frac{\sum_{\sigma^+}\sum_{\sigma^-} B_{\sigma^+,\sigma^-}}{\sum_{\sigma^+}\sum_{\sigma^-} A_{\sigma^+,\sigma^-}} \tag{S6}$$

is a function of parameters U_b, U_a, U, μ_p, μ_r and T. The free energy per lattice site is

$$\mathcal{F} = -\frac{k_{\rm B}T}{N}\ln \mathcal{Z}_{\rm MF} = 2\varepsilon - k_{\rm B}T\ln\Big[\sum_{\sigma^+=\pm1}\sum_{\sigma^-=\pm1} A_{\sigma^+,\sigma^-}\Big] + \mathcal{F}_0 \tag{S7}$$

where

$$\mathcal{F}_0 = -\frac{k_{\rm B}T}{N}\ln\left\{\Big[\prod_i\int_0^\infty {\rm d}l_i\Big] e^{-\beta\left[\mathcal{H}_{\rm me}+\sum_i V_{\rm b,eff}(l_i)\right]}\right\} \tag{S8}$$

is the free energy per lattice site for the reference system of two *homogeneous* membranes with Hamiltonian $\mathcal{H}_0 = \mathcal{H}_{\rm me} + \sum_i V_{\rm b,eff}(l_i)$.

Phase separation occurs if $\mathcal{F}$ exhibits two equal minima separated by a maximum, implying that $\partial\mathcal{F}/\partial s = 0$ has three roots, and $\partial^2\mathcal{F}/\partial s^2$ is negative for one of the roots and positive for the other two. The condition $\partial\mathcal{F}/\partial s = 0$ leads to the self-consistent equation

$$\begin{aligned} s &= P_{\rm b}\frac{\sum_{\sigma^+}\sum_{\sigma^-}\frac{1}{2}(\sigma^+ + \sigma^-)B_{\sigma^+,\sigma^-}}{\sum_{\sigma^+}\sum_{\sigma^-}B_{\sigma^+,\sigma^-}} + \\ &\quad (1-P_{\rm b})\frac{\sum_{\sigma^+}\sum_{\sigma^-}\frac{1}{2}(\sigma^+ + \sigma^-)A_{\sigma^+,\sigma^-}}{\sum_{\sigma^+}\sum_{\sigma^-}A_{\sigma^+,\sigma^-}} \end{aligned} \tag{S9}$$

where $P_{\rm b} = -\partial\mathcal{F}_0/\partial U_{\rm b,eff} = \langle\theta(l_b/2-|l_i-l_c|)\rangle_{\mathcal{H}_0}$ is the so-called contact probability of the *homogeneous* membranes. More precisely, $0 \le P_{\rm b} \le 1$ is the expectation value for the fraction of bound membrane patches, i.e., membrane patches with $l_c - l_b/2 < l_i < l_c + l_b/2$ in the reference system.

In the case of two planar membranes within the R-L binding range, i.e. with $\theta(l_b/2-|l_i-l_c|) = 1$ at any site i, $P_{\rm b} = 1$. Eq. (S9) reduces to

$$s = \frac{\tilde{f}_{1,1}e^{2\beta Us} - \tilde{f}_{-1,-1}e^{-2\beta Us}}{\tilde{f}_{1,1}e^{2\beta Us} + \tilde{f}_{-1,-1}e^{-2\beta Us} + 2f_{-1,1}} \tag{S10}$$

with $f_{\sigma^+,\sigma^-} = 1 + e^{\beta(\frac{\sigma^++1}{2}U_a+\mu_p)} + e^{\beta(\frac{\sigma^-+1}{2}U_a+\mu_p)} + e^{\beta(\frac{\sigma^++\sigma^-}{2}U_a+U_a+2\mu_p+U_b)}$, $\tilde{f}_{1,1} = f_{1,1}e^{\beta(\mu_r+2U)}$, and $\tilde{f}_{-1,-1} = f_{-1,-1}e^{-\beta(\mu_r+2U)}$. The free energy in Eq. (S7) becomes

$$\mathcal{F} = Us^2 - U - \mu_r - k_{\rm B}T\ln\left(\tilde{f}_{1,1}e^{2\beta Us} + \tilde{f}_{-1,-1}e^{-2\beta Us} + 2f_{-1,1}\right) \tag{S11}$$

Evidently, Eq. (S10) has one trivial root $s = 0$, and two other roots opposite in signs and yielding the same free energy when $\tilde{f}_{1,1} = \tilde{f}_{-1,-1} = (f_{1,1} f_{-1,-1})^{1/2}$, i.e., $\mu_r = -2U - k_B T \ln t$ with $t = (f_{1,1}/f_{-1,-1})^{1/2}$. This leads to the phase transition line

$$U = -k_B T \frac{1}{2s} \ln \frac{(1 - s^2 + r^2 s^2)^{1/2} - rs}{1 + s} \tag{S12}$$

with $r = f_{-1,1}/(f_{1,1} f_{-1,-1})^{1/2}$. The lowest point on this line gives the critical contact energy U^*. The area concentration of the adhesion proteins on each membrane, $c_p = -\left(\partial \mathcal{F}/\partial \mu_p\right)/(2a^2)$, is given by

$$a^2 c_p = 1 - \frac{(e^{2\beta U s}/t + 1)\left[1 + e^{\beta(U_a + \mu_p)}\right] + (e^{-2\beta U s} t + 1)(1 + e^{\beta \mu_p})}{\tilde{f}_{1,1} e^{2\beta U s} + \tilde{f}_{-1,-1} e^{-2\beta U s} + 2 f_{-1,1}} \tag{S13}$$

Eqs. (S12) and (S13) together determine the phase diagram in the (c_p, U) coordinates for the planar membrane system.

In the case of fluctuating membranes we determined P_b by simulating the reference system of two homogeneous membranes with Hamiltonian $\mathcal{H}_0$. We then identified the phase transition points by numerically solving Eq. (S9) under the same constraint as for the planar membrane system that the solutions shall lead to equal minima of the free energy $\mathcal{F}$.

To determine the Hill plots, i.e. the plots of $\log([RL]/[R])$ *versus* $\log([L])$, we first determined the chemical potentials μ_r and μ_p via the relations $c_p = -\left(\partial \mathcal{F}/\partial \mu_p\right)/(2a^2)$ and $x = -\left(\partial \mathcal{F}/\partial \mu_r\right)/(2a^2)$ for given values of c_p, x, U_a, U_b, and U. We then obtained $[RL] = \left(\partial \mathcal{F}/\partial U_b\right)/a^2$, and $[R] = [L] = c_p - [RL]$. Here, the free energy per lattice $\mathcal{F}$ is given by Eq. (S7).

Supplementary figures

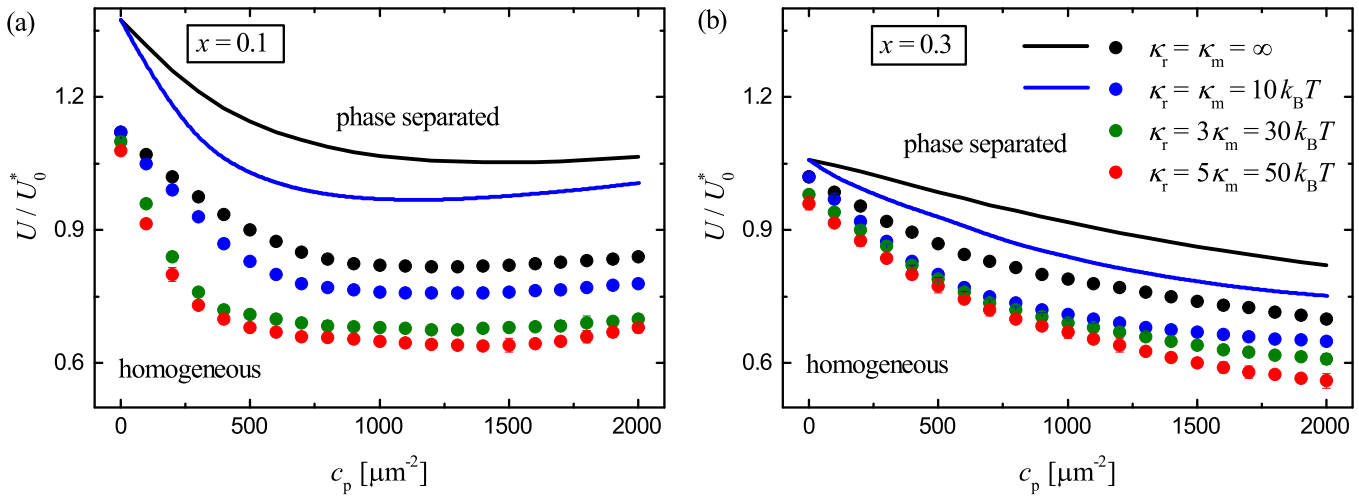

Fig. S1. Analogous to the phase diagram in Fig. 2d but for $x = 0.1$ (a) and $x = 0.3$ (b).

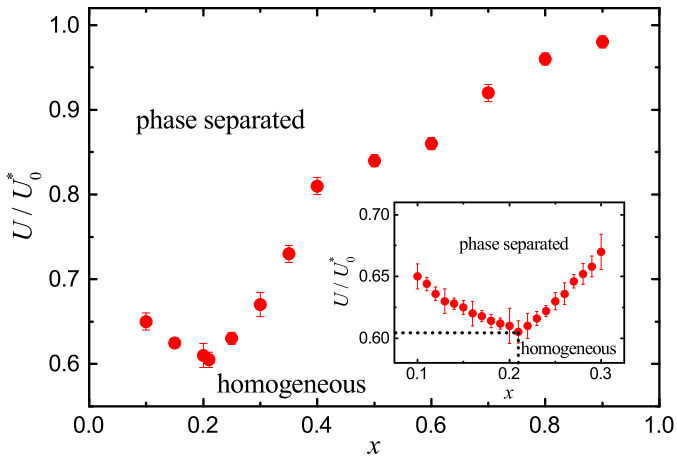

Fig. S2. An example of the phase diagram in the $(x, U/U_0^*)$ coordinates for $U_b = 6\,k_BT$, $U_a = 3\,k_BT$, and $c_p = 1000\,\mu m^{-2}$. The location of the critical point is indicated in the inset.

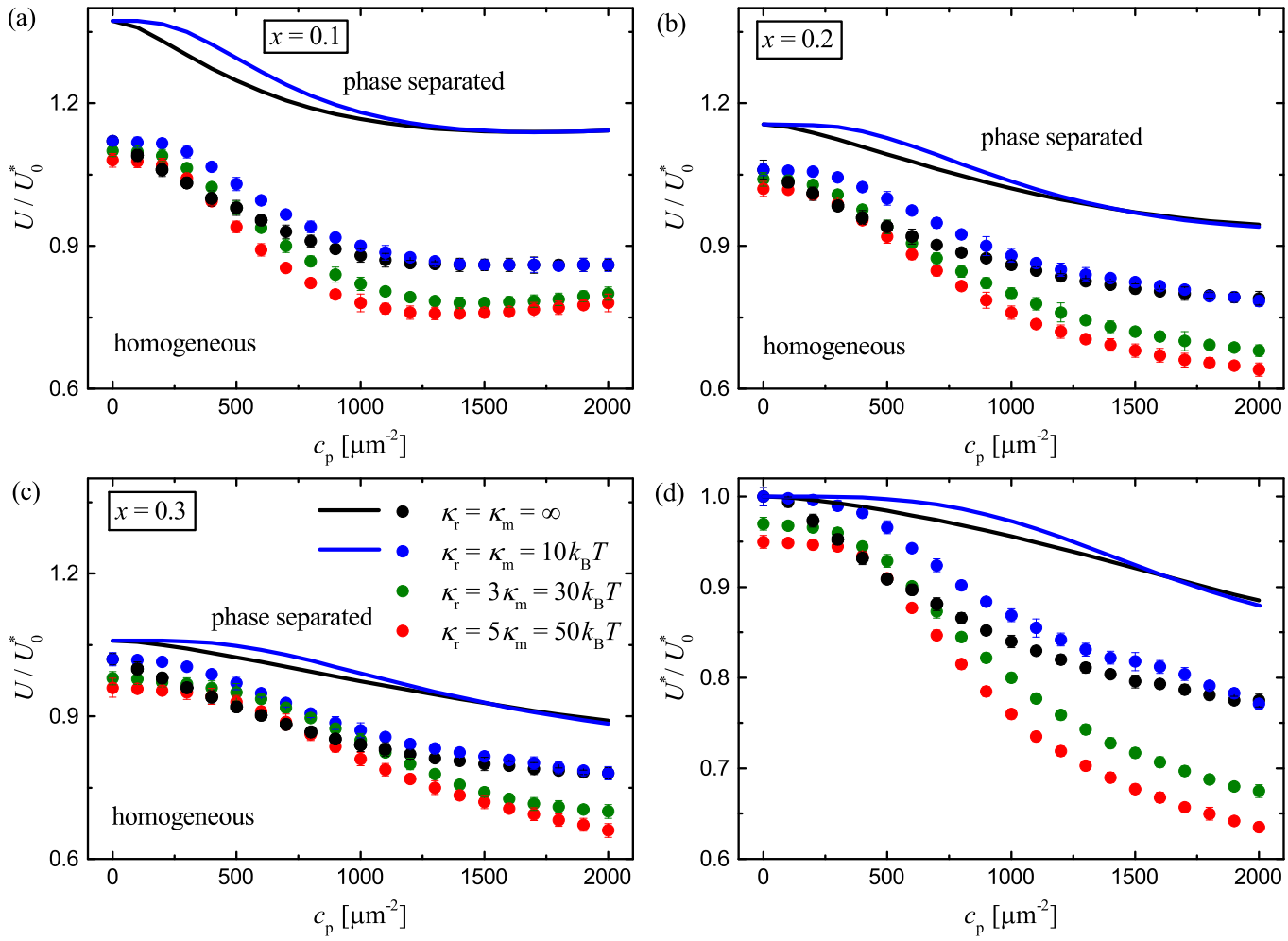

Fig. S3. MC and MF results for different membrane systems at $U_b = 3\,k_BT$, $U_a = 3\,k_BT$. (a)-(c) Phase diagrams in the $(c_p, U/U_0^*)$ coordinates for x=0.1 (a), 0.2 (b) and 0.3 (c). The bending rigidities κ_m and κ_r are indicated by different colors and specified in panel (c). The ordered and disordered phase is indicated in the phase diagrams. (d) Contact energy at the critical point, U^*, as a function of protein concentration c_p for membrane systems with different bending rigidities κ_m and κ_r. Color code as in panel (c).

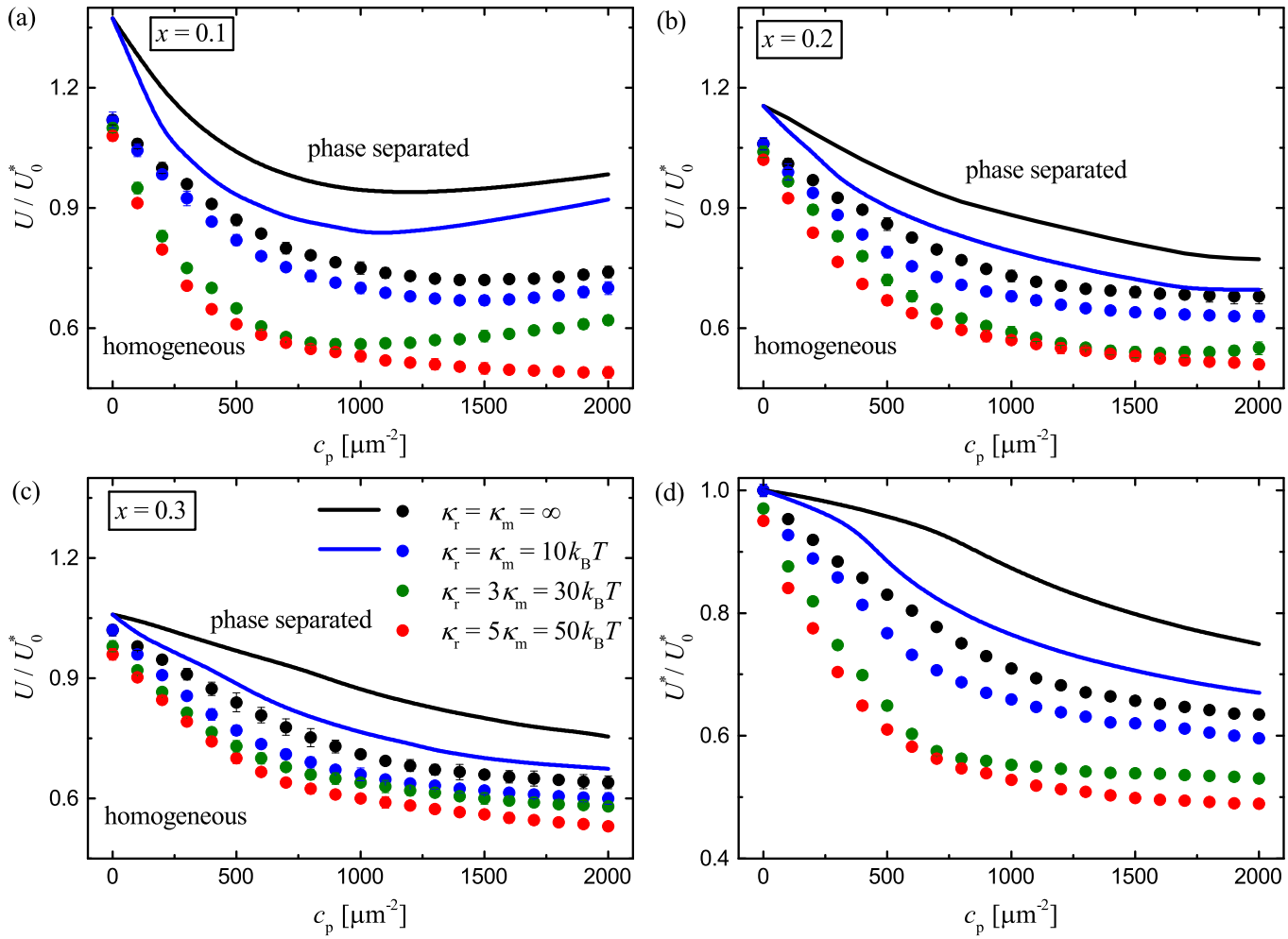

Fig. S4. Analogous to Fig. S3 but for $U_\mathrm{b} = 6\,k_\mathrm{B}T$ and $U_\mathrm{a} = 4\,k_\mathrm{B}T$.

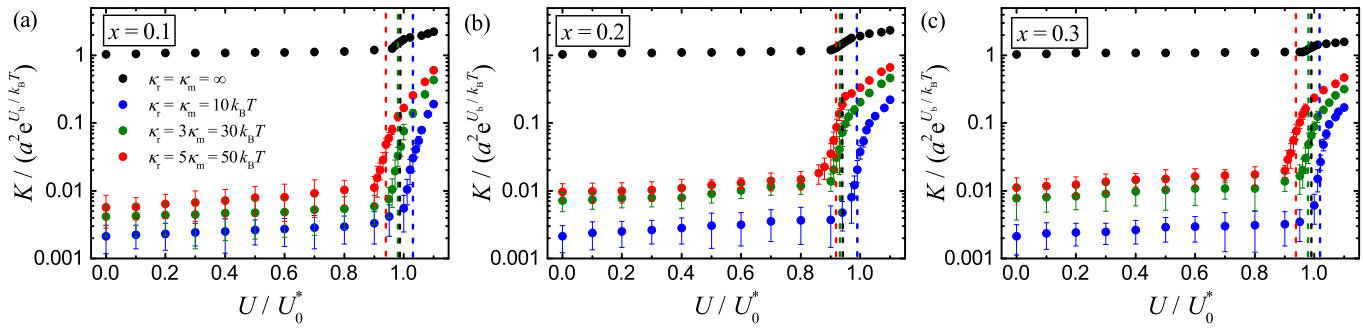

Fig. S5. Analogous to Fig. 3 in the main text but for $U_\mathrm{b} = 3\,k_\mathrm{B}T$ and $U_\mathrm{a} = 3\,k_\mathrm{B}T$.

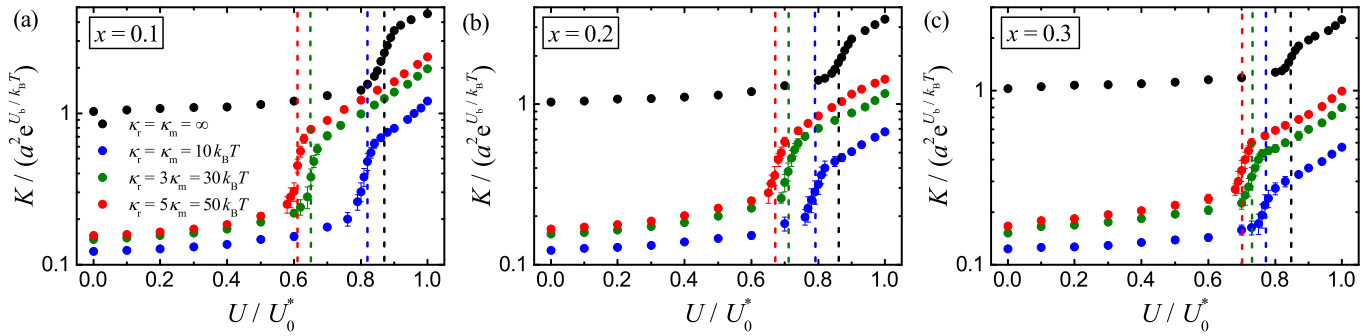

Fig. S6. Analogous to Fig. 3 in the main text but for $U_\mathrm{b} = 6\,k_\mathrm{B}T$ and $U_\mathrm{a} = 4\,k_\mathrm{B}T$.

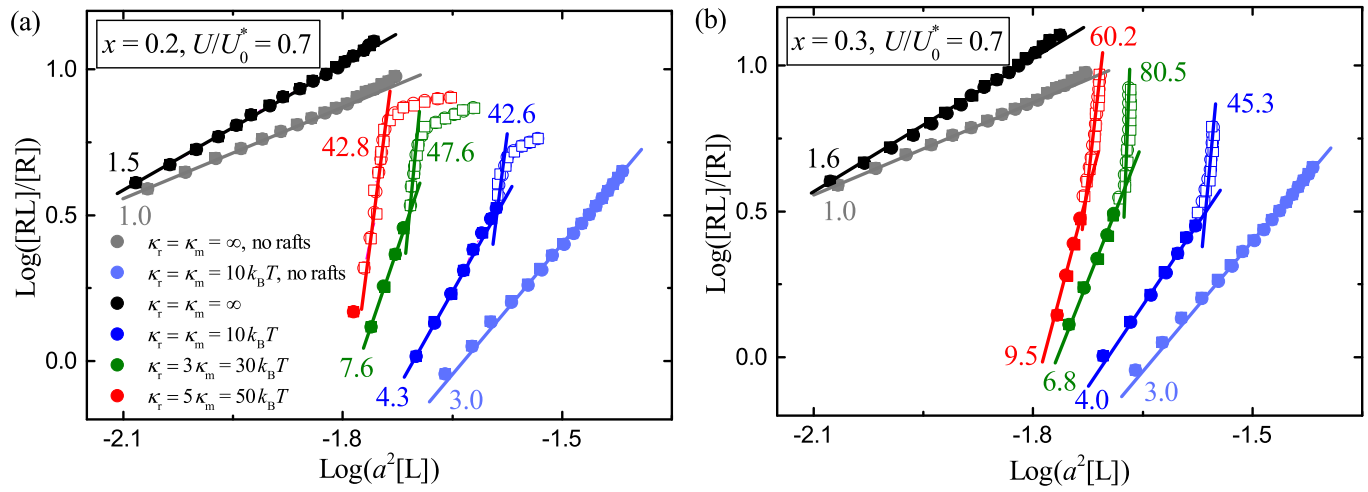

Fig. S7. Analogous to Figs. 4c and 4d in the main text but for different sizes of the adhering membranes. The data points marked as dots were obtained form MC simulations with 100×100 lattice sites. The data points marked as squares were obtained form MC simulations with 60×60 lattice sites.

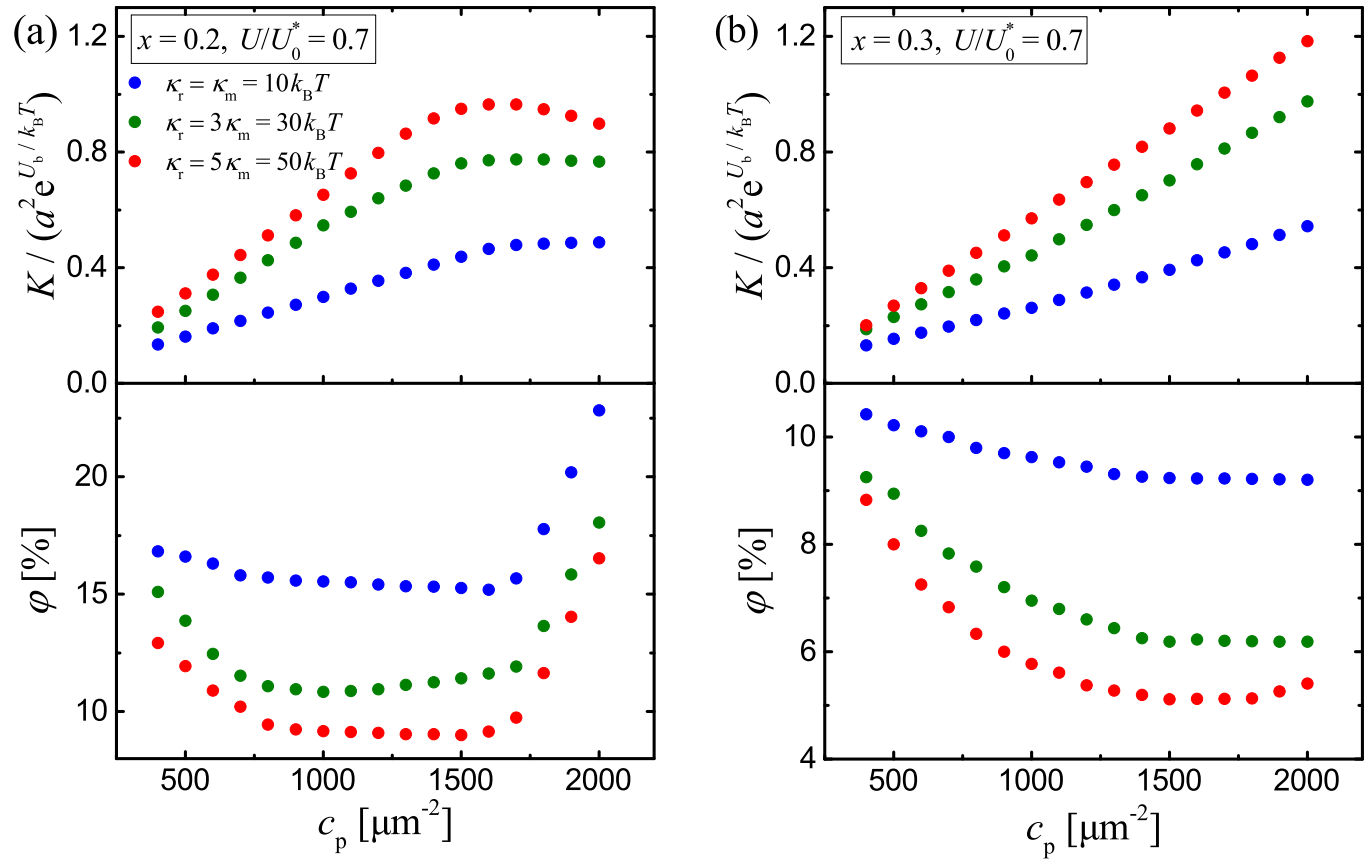

Fig. S8. Binding constant K and percentage of adhesion proteins in the membrane matrix, φ, as a function of protein concentration c_p. Here, $U_b = 6\,k_BT$, $U_a = 3\,k_BT$, $U = 0.7\,U_0^*$ and $x = 0.2$ (a) or $x = 0.3$ (b). The bending rigidities κ_m and κ_r of the membrane matrix and lipid rafts are specified in panel (a).