Lipid-raft phase modulation by membrane-anchored proteins with inherent phase separation properties

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Supporting Figures

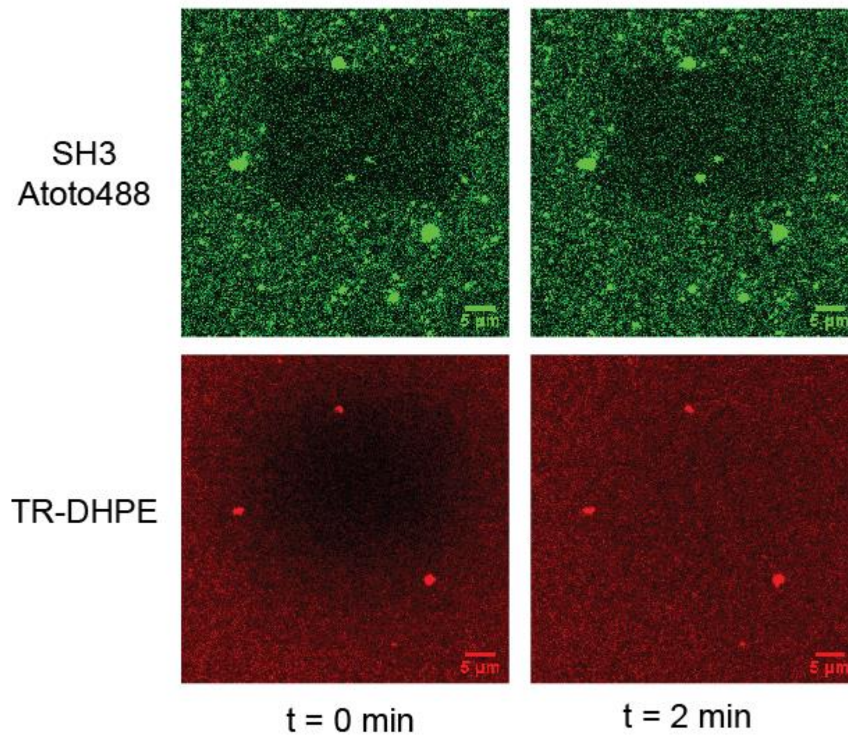

Figure S1. Fluorescence recovery after photobleaching (FRAP) shows partially mobile lipid membrane after sequential incubation of SH3x4 and PRMx4 on supported lipid bilayer. (SLB) SLB composition was 98% DOPC, 2% Ni-NTA and 0.01% TR-DHPE. 2 µM SH3x4 with polyhistidine tag was incubated for 30 min and washed with buffer. 2 µM of PRMx4 was introduced and incubated for 30 min. After the incubation FRAP was performed to check fluidity of the membrane. Protein molecules are only partially mobile, and unlinked lipid molecules are mostly fluidic shown by full FRAP recovery in 2 min. Small clusters were also visible on the biliayer which may have formed due to favorable multivalent binding between SH3x4 and PRMx4. Scale bars are 5 µm.

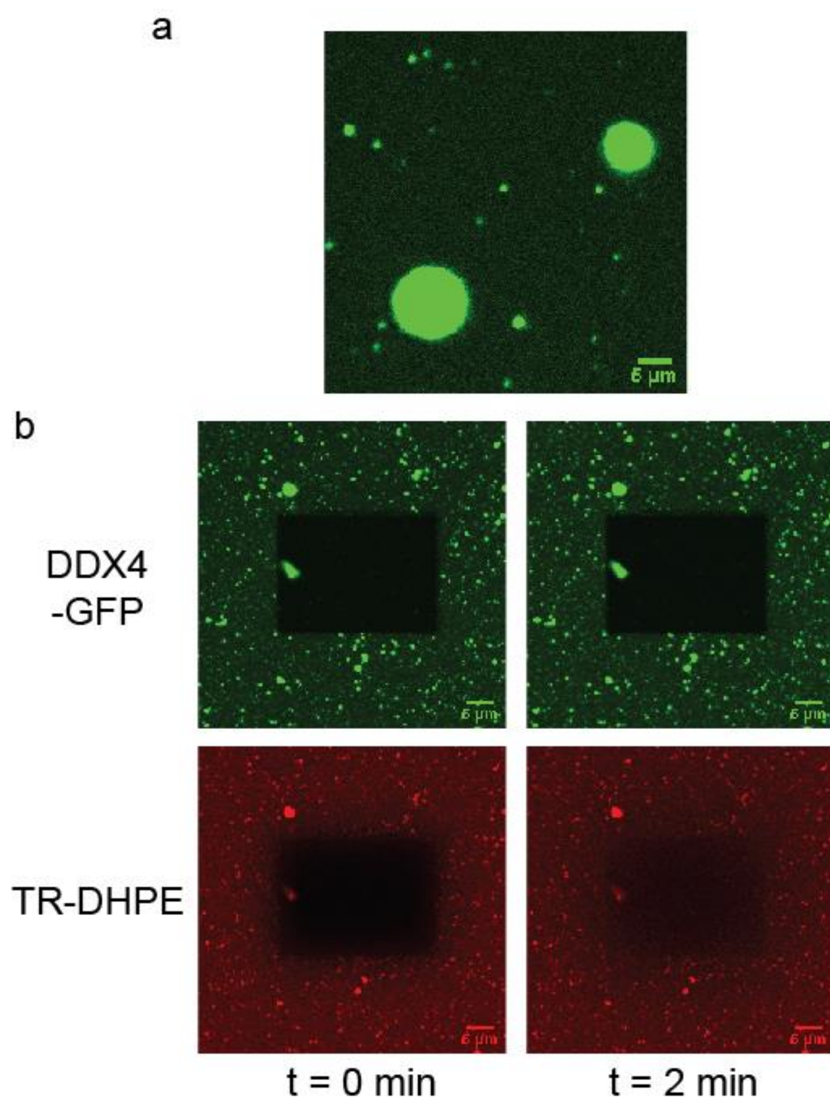

Figure S2. DDX4-GFP forming 3D droplets in solution and immobile lipid membrane on SLB (a) An example image of solution phase separated domains or droplets formed by DDX-GFP at high concentration > 100 µM (b) FRAP recovery of SLB after 30 min incubation with 1 µM for 30 min. SLB composition was 90% DOPC, 10% Ni-NTA and 0.01% TR-DHPE. DDX4-GFP showed almost no recovery and TR-DHPE channel recovered partially. Small clusters were also visible on the biliayer which may have formed due to favorable interaction of DDX4-GFP. Scale bars are 5 µm.

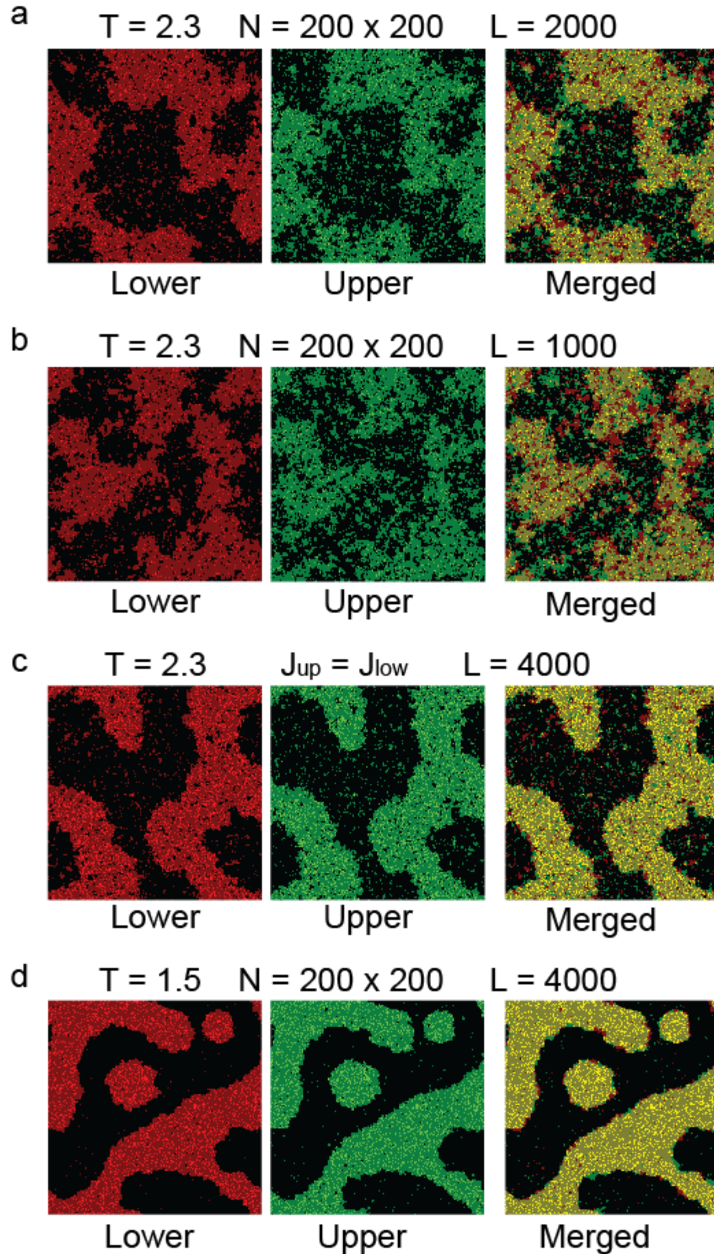

Figure S3. Additional Ising model simulation data show more coupling between two layers generates more enhanced and correlated phase separation. Spin states of a simulation after t = 20,000 steps. For lower layer, black and red colors indicate two spins. For upper layer, black and green colors indicate two spins. T = 2.3 was the critical temperature for the lower layer and the upper layer was above its critical temperature due to lower inter-particle stabilization energy (0.9 of the lower layer) for all simulations except c where both layers had equal stabilization energy J. N, or the total number of spins were 40,000 for each layer. (a) Spin states for T = 2.3, L = 2000. 5% of spins were linker spins and the system still showed strong correlation and enhanced phase separation. (b) Spin states for T = 2.3, L = 1000. 2.5 % of spins were linker spins and the system still showed strong correlation and enhanced phase separation. As L lowers, we saw less dense phase segregation. (c) Spin states for T = 2.3, L = 4000 with equal energetic stabilization J for

both layers. Correlated movement and enhanced phase separation could be observed. As J_{upper} is higher than other simulations (1.0 instead of 0.9 J_{lower}), we could see further enhanced phase separation behavior (d) Spin states for T = 1.5, L = 4000. At temperature far below the critical temperature, denser phase separation was observed as expected and two layers were very strongly correlated.

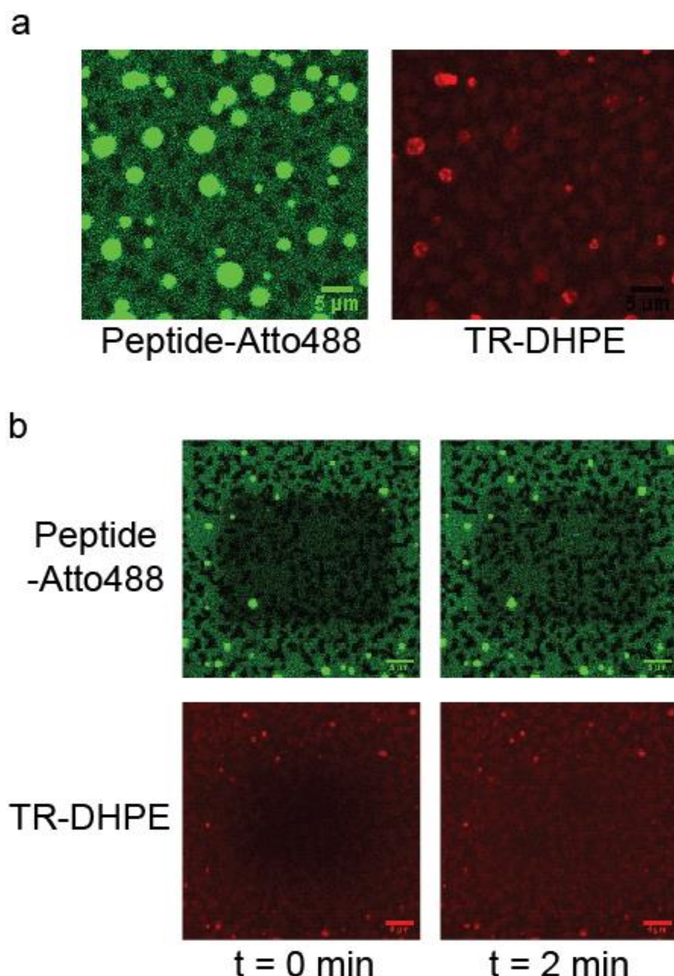

Figure S4. Three-component protein system on the supported lipid bilayer. (a) Three component system forms a network of protein layer on the membrane, and 3D phase droplets tend to smear on the membrane forming circular domains which were at least an order of magnitude concentrated than proteins on the membrane. (b) Fluorescence recovery after photobleaching (FRAP) shows pretty mobile lipid membrane after the incubation. Protein channel recovered > 50% and lipid channel recovered nearly 100% within 2 min after photobleaching. SLB composition was 98% DOPC, 2% Ni-NTA and 0.01% TR-DHPE. All three proteins were originally mixed as 25 µM each in buffer which readily formed phase droplets. The solution was then diluted into 5 µM to interact with the SLB. The images were taken after 30 min of incubation.

Supporting Methods

DDX4-GFP gene sequence

The yellow colored region is from the IDR of DDX4 and the green colored region is for GFP

MGDEDWEAEI NPHMSSYVPI FEKDRYSGEN GDNFNRTPAS SSEMDDGPSR RDHFMKSGFA SGRNFGNRDA GECNKRDNTS TMGGFGVGKS FGNRGFSNSR FEDGDSSGFW RESSNDCEDN PTRNRGFSKR GGYRDGNNSE ASGPYRRGGR GSFRGCRGGF GLGSPNNDLD PDECMQRTGG LFGSRRPVLS GTGNGDTSQS RSGSGSERGG YKGLNEEVIT GSGKNSWKSE AEGGES C ENLYFQG M V S K G E E L F T G V V P I L V E L D G D V N G H K F S V S G E G E G D A T Y G K L T L K F I C T T G K L P V P W P T L V T T L T Y G V Q C F S R Y P D H M K Q H D F F K S A M P E G Y V Q E R T I F F K D D G N Y K T R A E V K F E G D T L V N R I E L K G I D F K E D G N I L G H K L E Y N Y N S H N V Y I M A D K Q K N G I K V N F K I R H N I E D G S V Q L A D H Y Q Q N T P I G D G P V L L P D N H Y L S T Q S K L S K D T N E K R D H M V L L E F V T A A G I T L G M D E L Y K HHHHHHHHHHHH

LAT sequence

HHHHHHHHHHGGSGGSGGD{pY}VNVGGSGGSGGD{pY}VNVDKKC

SLB protocol

Small unilamellar vesicles (SUV) were made by extrusion. Net 2 mg of lipid mixture in chloroform solution was dried on a clean round bottom flask surface by 30 – 45 min of rotavap drying at 50 °C. The dried lipid film was further dried by 2-5 min of Nitrogen blow. 1 ml of Milli-Q water was added to perform three times of freeze thawing. The sample was completely frozen by lowering temperature of the sample by dry ice then was thawed by raising the temperature in water bath. Completely hydrated lipid solution was then extruded nine times through 100 nm pore extrusion sheet. Nanosizer extrusion kit from T&T scientific was used. Collected SUV solution was kept at 4 °C to use within a week.

To prepare SLB, round cover glasses (Fischer Scientific) were cleaned by bath sonication for 30 min in isopropyl alcohol:H_2O = 1:1 v/v solution. After multiple rinsing, the cover glasses were piranha etched for 2 - 5 min in acid piranha solution. (Sulfuric acid:H_2O_2 solution = 3:1 v/v) The cover glasses were thoroughly rinsed with Milli-Q water and then used within 24 hours. One of the cover glasses was assembled into the home built stailess steel sample chamber unit, and SUV with net 0.4 mg of lipids was incubated on top of the glass in 1x PBS 2-5 mM $MgCl_2$. After 45 min incubation for spontaneous SLB formation, the solution above SLB was washed by exchanging buffer >5 times without exposing the lipid surface in the air. Optionally, it was further incubated with 100 mM $NiSO_4$ solution for 5 min to completely charge Ni-NTA lipids with Ni^{2+} ions.

Ising model simulation

1. Initialization

Two layers of 200 by 200 spins, represented by two 200 by 200 number matrices were initialized by randomly mixing up-spins and down-spins at high temperature. Spins were randomized without changing the total number of up-spins and down-spins in each layer. There were total 40,000 spins in each layer and 20,000 of them were up-spins and another 20,000 of them were down-spins. L of linker-spins were assumed where spins in two layers at the same position (x, y coordinate) were coupled, so the spin could only move by also moving its coupled spin on the other layer. Two coupled spins in two layers were both assumed to be all up-spins. Linker-spin positions were also randomized in the initialization.

2. Spin exchange simulation (a single step)

2a) One spin was chosen randomly.

2b) A random movement was decided randomly. It was one of four possible movements. ± 1 in x coordinate or ± 1 in y coordinate. A movement means two spins will exchange positions effectively moving the chosen spin toward that direction. Spins were not allowed to move between two layers. Periodic boundary condition was assumed for both layers.

2c) ΔE of the proposed movement was calculated according to the following equation.

ΔE = - J × [{(# of neighboring identical spins after movement) – (# of neighboring different spins after movement)} –{(# of neighboring identical spins before movement) – (# of neighboring different spins before movement)}]

J = 1.0 for lower layer and J = 0.9 for upper layer. By the unitless definition we used in our simulations, it means at T = 2.3, the lower layer was at its original critical temperature (T_C of the system is 2.3 when J = 1.0 assuming a single layer 2D Ising model), but the upper layer was still above its original transition temperature or was at homogeneous temperature. Neighboring spins were only considered in xy coordinates and two spins at the same position of two layers were not considered as neighboring spins. (z coordinate neighboring was not considered) This was to assume the experimental situation where proteins and lipids affected each other only through anchors or there was no direct lipid-protein binding.

If the chosen spin was a linker-spin, simultaneous movement of spins at both layers was assumed and the difference in energy was also calculated accordingly.

2d) The movement was accepted or rejected based on ΔE. When $\Delta E < 0$, the movement was accepted and spins were exchanged. When $\Delta E \geq 0$, a random number was generated to make a decision. The movement was accepted only when the random number was smaller than the exp(-ΔE/T), and the movement was rejected when it was larger than the probability. It naturally increased the chance of movement at higher temperature. When the movement was rejected, no spin exchange was performed.

2e) 2a to 2d was repeated $2 \times 40{,}000$ times for a unit step. A unit step was a minimal unit of a reaction time in simulation.

3. Continued dynamic simulation

Step 2 was repeated for desired amount of time to reach an equilibrated and a representative state of the system at the simulated temperature. t = 2,000,000 steps calculation was performed for each simulation which was enough to reach the representative state of the system. Spin states of both layers were recorded at every 100,000 step to closely monitor the change of states.

4. Analyzing the result

After ensuring that the final spin state at t = 2,000,000 was a representative state, the final state was used to interpret the result of the simulation.