Membrane Obstacles Enhance the Sensitivity and Selectivity of Surface Condensation

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Biomolecular phase separation has emerged as an essential mechanism for cellular organization. How cells respond to environmental stimuli in a robust and sensitive manner to build functional condensates at the proper time and location is only starting to be understood. Recently, lipid membranes have been recognized as an important regulatory center for biomolecular condensation. However, how the interplay between the phase behaviors of cellular membranes and surface biopolymers may contribute to the regulation of surface condensation remains to be elucidated. Using simulations and a mean-field theoretical model, we show that the presence of protein obstacles at physiological concentrations in the membrane enhances the sensitivity and selectivity of surface condensation formation and membrane reorganization to the property of the biopolymer. Such obstacle effects are robust over a range of membrane conditions, and originate from the positive co-operativity between the coupled growth of local lipid domains and biopolymer condensates. The general physical principle emerged from the current analysis may have implications in other biological processes and beyond.

phase separation | membrane reorganization | protein obstacles | surface condensation | co-operativity

Cells are compartmentalized into distinct functional regions often surrounded by biological membranes, known as organelles, for carrying out the diverse biochemistry of life. In addition, phase separation driven by weak, multivalent interactions among biomolecules has emerged as an essential mechanism for cellular compartmentalization (1). The biomolecular condensates formed through phase separation, enriched in selected proteins and often RNAs, are known as membraneless organelles and have been revealed to play an essential role in cell physiology (2–6). Unlike membrane-bound organelles, the formation of these phase-separated condensates are typically reversible in response to cellular cues (7–12). The mechanism of how cells respond to stimuli in a robust and sensitive manner to build specific functional condensates in a spatially and temporally relevant manner is only starting to be understood.

In recent years, biological membranes have been recognized as a key regulatory center for controlled condensate formation in cells (13–17). In particular, prewetting appears to be a mechanism by which cells could exert spatiotemporal control over the assembly of biomolecular condensates (18–20). In general, proteins separate into a coexisting dense phase (condensate) and a dilute phase in the cytoplasm when their mutual interaction strength reaches a certain threshold value J_c. In a prewetting process, proteins (or biopolymers in general) are attracted to a surface, which enables their condensation at interaction strengths lower than J_c. This surface condensate (prewetting phase) is restricted only to the vicinity of the surface, as bulk condensation is not favorable. Yet beyond merely serving as an attractive two-dimensional surface, biological membranes are fluidic structures with heterogeneous and complex lipid and protein compositions that can phase separate on their own (21–24). The goal of this work is to understand the interplay between the phase behavior of biological membranes and the biopolymers at the membrane surfaces. We establish general principles for how cells regulate surface condensation.

In an interesting paper, Machta and co-workers studied the coupled phase behavior of a 2-component membrane and polymers; they focused on the conditions of coexistence of different surface phases and presented a framework for analyzing the problem through simulation and theory (18). The main conclusion of their work was that proximity to the bulk critical point plays an important role in condensate formation. An important feature of biological membranes that their work did not include is the presence of membrane proteins. Various experimental and theoretical studies have estimated that the area fraction of proteins in biological membranes ranges from 20 to 75% depending on the membrane type (25–31), and that these embedded "protein obstacles" have significant influences on the phase behaviors of the surrounding lipids (32–34). Therefore, a realistic model for studying the coupled phase behaviors of membrane and surface biopolymers must take protein obstacles into account (Fig. 1).

In this work, we explore the effect of membrane protein obstacles on regulating surface condensation through grand canonical Monte Carlo (GCMC) simulations and a mean-field theory (MFT). Our simulation results show that the presence of protein obstacles in the membrane at physiological concentrations enhances the sensitivity and selectivity of surface condensation formation and membrane reorganization to the property of the biopolymer, and that such effect is observed over a range of membrane conditions. Furthermore, our theoretical analysis confirms the findings of the simulations, and reveals that such obstacle effect originates from the positive co-operativity between the coupled growth of local lipid domains and surface condensates. Although the simple model used here does not represent the rich membrane chemistry present in biology, the underlying physical principles are robust and potentially relevant to many biological processes.

Simulation Results

Simulation Model for the Effect of Protein Obstacles on Membrane Phase Behaviors. Before exploring the effect of obstacles on surface condensation, we first briefly review the basic simulation framework and the effect of obstacles on the phase

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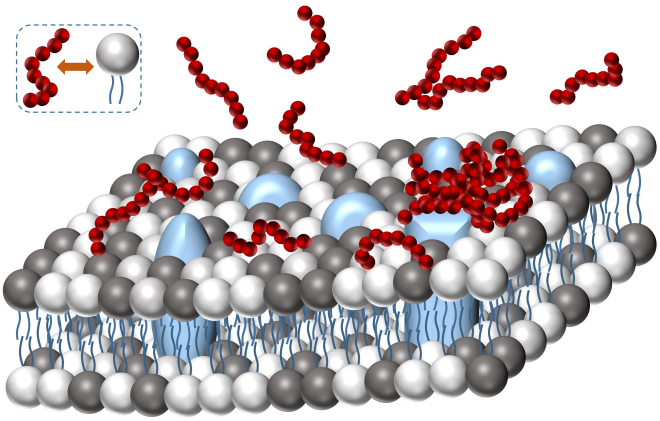

Fig. 1. Schematic of biopolymers (red) at the surface of a membrane consisting of two types of lipids (black and white) and embedded protein obstacles (blue), where biopolymers are attracted to one type of lipid (white). How the obstacles modify the coupled phase behavior of biopolymers and lipids to regulate surface condensation is the question under investigation.

behavior of membrane. We model binary lipid membrane embedded with protein obstacles using a fixed composition 2D Ising model on square lattice with an attractive/repulsive nearest neighbor (NN) interaction energy $J_m(k_BT)$ between like/unlike lipid pairs (see *SI Appendix*, Fig. S1 A). The Hamiltonian of the system is

$$H_{Ising} = -J_m \sum_{i,j \in NN} S_i S_j, \qquad [1]$$

where i and j label lattice sites, $S_i = 1/-1$ for lipid species A/B and $S_i = 0$ if it's occupied by an obstacle, indicating that obstacles are inert and have no (preferential) interaction with either lipid. In the simulations, J_m and the fraction of obstacle sites (f_o) are varied to describe different membrane conditions, while the number of A lipid is always kept equal to that of B ($M = \sum_i S_i = 0$), representing fixed lipid compositions. Furthermore, we consider two types of obstacles: immobile and mobile (floating). Immobile obstacles describe integral membrane proteins attached to the cytoskeleton or very large integral membrane proteins that diffuse much slower than lipids, while mobile obstacles describe unattached floating membrane proteins. In a simulation with immobile obstacles, the positions of obstacle sites are fixed at initialization and the only MC move is the swap of unlike lipid pairs, while in simulations with mobile obstacles, swap moves between lipids and obstacles are included. We refer to immobile/mobile obstacles as obstacles/floating obstacles hereafter for simplicity. Our simulations show that the presence of obstacles significantly suppresses lipid phase separation, in agreement with previous studies (32–34) (see *SI Appendix* and Fig. S1-3 for detailed discussions). For example, as the fraction of obstacles increases from 0 to 0.1 and 0.3, the critical membrane coupling J_m^c increases from 0.35 k_BT to 0.4 and 0.7 k_BT, respectively (Fig. S1C-E). The effect of floating obstacles, however, is far less prominent (Fig. S2).

Membrane obstacles enhance the sensitivity and selectivity of surface condensation. We next explore the effect of membrane obstacles on surface condensation by coupling the Ising membrane with lattice polymers in our simulation (Fig. 2*E*). Specifically, the Ising membrane is simulated as described in the previous section and placed at the bottom of the simulation box ($z = 0$). Lattice polymers are 9 monomers long with attractive NN interaction energy $J_p(k_BT)$, and are kept at fixed chemical potential following the conventional GCMC algorithm (35). Following Rouches et al. (18), the polymers are coupled to the membrane through tethers, which are attached only to lipid species A and extend 5 lattice sites straight into the bulk. Tethers form favorable interactions with polymers, and can translate on the 2D membrane. It should be noted that the inclusion of tethers here is not essential, and is merely one way of implementing attraction between polymers and selected lipid species, without which the membrane phase behavior does not affect surface condensation. The major conclusions from this study are not subject to specific forms of polymer-lipid coupling and apply more generally, as further discussed in the MFT section.

Without the membrane, the lattice polymers can phase separate in the bulk when J_p is increased to 0.55 k_BT (see *SI Appendix*, Fig. S4A and B), and thus we focus on $J_p < 0.55$ k_BT, where the surface condensates formed are prewetting phases. Typical surface condensates formed in prewetting are shown in the simulation snapshots in Fig. 2*E* and *SI Appendix* Fig. S4C, which are thin layers of polymer aggregates (see *SI Appendix* Fig. S5E). Indeed, when J_p reaches 0.55 k_BT, surface condensates start to grow into the bulk (see *SI Appendix*, Fig. S4D).

Next, we analyze how the assembly of surface condensate responds to the change of J_p under different membrane conditions. The change of J_p is meant to be a simple model for the variation of biopolymer sequence, the state of post-translational modification (e.g., phosphorylation) or local solution condition (e.g., pH), which are regulatory mechanisms that cells exploit to control biomolecular phase separation (36).

As shown in Fig. 2*A*, without obstacles, the density of the surface condensate formed increases with both J_m and J_p, as manifested by the deeper color towards the lower right of the heat map, whereas, at high J_p values, the dependence on J_m is significantly weakened. Such qualitative trends remain in the corresponding heat maps after introducing obstacles (Fig. 2$B-D$), while high condensate densities are observed at higher J_p values as the amount of obstacles increases. It should be emphasized that a high condensate density is required for meaningful volume compartmentalization and component enrichment to form functional assemblies. In addition, a closer comparison between the condensate densities in Fig. 2$B-D$ and Fig. 2*A* reveals that their differences primarily reside at columns of intermediate J_p values (Fig. 2$F-H$), indicative of different response patterns of surface condensates to J_p with and without obstacles.

The change in response of surface condensation to J_p upon obstacle introduction becomes clearer by examining horizontal rows of Fig. 2$A-D$. As shown in Fig. 3*A*, at fixed J_m, without obstacles (see blue curve of Fig. 3*A*), the density of surface polymer aggregate remains low and slow-growing before J_p reach the threshold of 0.2 k_BT, after which the density increases more rapidly with J_p and saturates at $J_p = 0.45$ k_BT, representing an overall gradual response to J_p variation. With the introduction of membrane obstacles at fractions of 0.1, 0.2 and 0.3, the corresponding threshold J_p is progressively shifted

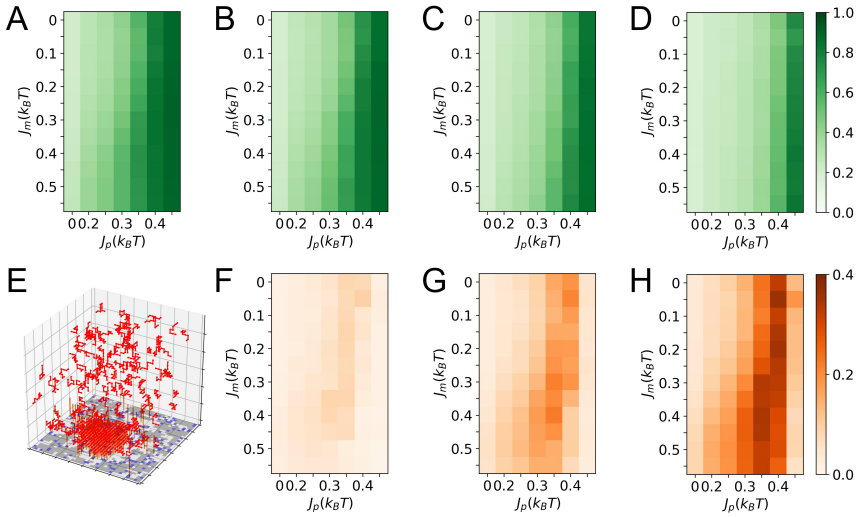

Fig. 2. Effect of obstacles on the densities of surface condensates. (A) Polymer density in surface condensates formed at different $J_p(k_BT)$ and $J_m(k_BT)$ without obstacles and with obstacles at area fractions of 0.1 (B), 0.2 (C) and 0.3 (D). (E) Snapshot of a simulation with J_p = 0.45 k_BT and J_m = 0.35 k_BT with floating obstacles at an area fraction of 0.1. Red chains represent polymers, white, grey and blue squares on the bottom plane represent the two lipid components A and B and protein obstacles of the membrane. Brown straight chains sticking out of the bottom plane represent tethers that only connect to white lipids and have a favorable interaction with the red polymers. (F-H) Polymer density differences between (A) and (B-D). Polymer density is calculated as the fraction of lattice sites occupied by red polymers in a $5 \times 5 \times 5$ surface region. The tether density of the membrane is $\rho_t = \frac{number\ of\ tether}{number\ of\ lipid\ A} = 0.2$ in all cases.

to higher values (see orange, green and red curves of Fig. 3*A*), which reflects enhanced selectivity towards polymers for the formation of dense surface condensates. Furthermore, the condensate density varies more steeply to increasing J_p as the amount of obstacles increases. For example, at the obstacle fractions of 0 and 0.3, condensate densities reach ~ 0.35 at $J_p = 0.2$ and 0.35 k_BT respectively; both densities increase to ~ 0.85 when J_p reaches 0.45 k_BT, showing a sensitivity enhancement by a factor greater than two due to the presence of membrane obstacles.

The presence of obstacles also contributes to regulating the accompanying membrane reorganization during surface condensation. As demonstrated in Fig. 3*B* and *C* (see also *SI Appendix*, Fig. S6), the effect of obstacles on the response of membrane composition beneath the surface condensate to J_p, in terms of the concentrations of lipid A and tether, are similar to that observed in Fig. 3*A* for polymer density, while featuring more significant sensitivity enhancements. It should be noted that the realization of sensitive membrane reorganization beneath surface condensation by obstacles is functionally significant. Biological membranes have been proposed to be consist of domains of distinct composition and properties for accomplishing various cellular functions (23, 37–40). However, direct evidence for the existence of large lipid domains *in vivo* have been lacking, which was proposed to be due to their context-dependent nature and the multiplicity of their possible organizational states (41, 42). In line with this, our simulation shows that while the presence of macroscopic lipid domains is suppressed by obstacles, local lipid domain assembly is facilitated by a contacting surface condensate (demonstrated in Fig. 3*D* and *E*). Thus, the broad existence of protein obstacles in biological membranes may ensure that local lipid domains form only when needed.

The observed obstacle effects persist over condensate sizes (see *SI Appendix*, Fig. S5), obstacle types (see *SI Appendix*, Fig. S7 and Fig. S8D) and lipid environments (reflected by different J_m values, see *SI Appendix*, Fig. S8A-C). To better understand the physical origin of such robust obstacle effects, we reason that the obstacles can affect the membrane in two ways relevant to surface condensation. First, obstacles suppress membrane phase separation. Second, the presence of obstacles reduces the effective tether density on the entire membrane by a factor of (1-f_o), especially in the lipid-mixed state of the membrane. Hence we set out to explore if we can reproduce the obstacle effects by repeating the obstacle-free simulations (blue curve of Fig. 3*A*) at reduced effective J_m and tether density (ρ_t). From such analysis, we conclude that the sensitivity and selectivity enhancing effect of obstacles on surface condensation originates from creating membrane conditions unfavorable for forming dense polymer aggregates (see *SI Appendix* and Fig. S9 for detailed discussions). To verify this observation, we further performed obstacle-free simulations (blue curve of Fig. 3*A*) at an increased tether density, which indeed resulted in a broader and slower response of condensate density to J_p (see pink curve of Fig. 3*A*).

Mean-field Theory for the Obstacle Effects

To gain a deeper understanding of the obstacle effects on surface condensation, we then developed a theoretical model for our simulation system. Specifically, the free energy for the formation of surface condensation (F) is divided into three contributions: 1. aggregation of polymers from the bulk to the surface condensate; 2. reorganization of lipids and tether in the membrane beneath the surface condensate; and 3. the formation of interaction between tethers and the condensate polymers. We then minimize F, with and without obstacles, at different J_p values with respect to the density of the surface condensate (ϕ_0) and the composition of the membrane underneath; these analyses enable us to dissect the effect of obstacles on the response of surface condensate and membrane composition to J_p.

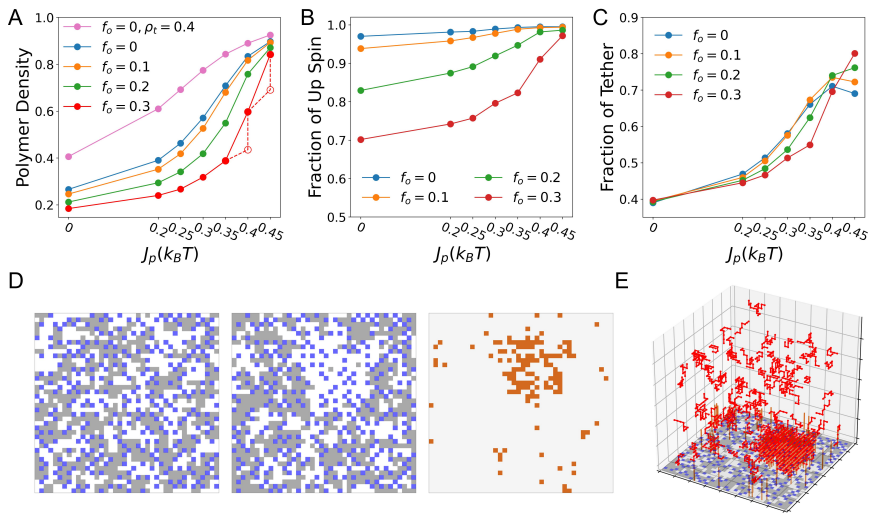

Fig. 3. Membrane obstacles enhance the sensitivity and selectivity of surface condensation and its complementary membrane reorganization to J_p. (A) Polymer density of surface condensate as a function of J_p at fixed J_m = 0.55 k_BT and $\rho_t = 0.2$ unless otherwise stated. The two empty red circles show data for $f_o = 0.3$. Specifically, the left (right) empty red circle shows condensate density achieved when J_p is increased from 0.35 (0.4) k_BT to 0.4 (0.45) k_BT with membrane configuration frozen. For example, in the simulation corresponds to the left empty red circle, while J_p is set to 0.4 k_BT, the membrane is fixed at representative membrane configuration selected from the trajectory of normal simulation at J_p = 0.35 k_BT. (B) Fraction of type-A lipid (up spin) in all lipids beneath the surface condensate as a function of J_p at fixed J_m = 0.55 k_BT and $\rho_t = 0.2$. (C) Fraction of tether attached type-A lipid in all type-A lipids beneath the surface condensate as a function of J_p at fixed J_m = 0.55 k_BT and $\rho_t = 0.2$. f_o and ρ_t represent the area fraction of membrane obstacles and the overall concentration of tether on type-A lipids in the simulations. (D) (Left) Snapshot of membrane simulation at J_m = 0.35 k_BT with obstacle fraction of 0.2. (Middle) Snapshot of membrane in a simulation at J_p = 0.45 k_BT and J_m = 0.55 k_BT with obstacle fraction of 0.2. (Right) Corresponding tether distribution of the Middle snapshot. Chocolate and light grey squares represent positions with and without tethers. (E) Snapshot of the entire simulation system corresponding to the snapshot in D (Middle).

Adapted Flory-Huggins Free Energy of Polymers Captures the Bulk Simulation Result. We describe the free energy of the polymers with an adapted Flory-Huggins theory (43, 44), in which the average free energy per monomer in the bulk (f_9) is given by:

$$f_9 = \frac{1-\phi}{\phi}\ln(1-\phi) - \frac{1}{L}\ln(\frac{L}{\phi}) - \frac{1}{L}\ln(C_9) - \phi\frac{Z-2}{2}J_p. \quad [2]$$

Here ϕ is the polymer concentration, $Z = 6$ is the cubic lattice coordination number, and $L = 9$ and $C_9 = 193,983$ are the length and the number of conformations with a given starting site of a single 9-monomer chain in the bulk (see *Methods* and *SI Appendix* for details). As plotted in Fig. 4*A*, at low J_p values, f_9 increases monotonically with ϕ, indicating the dominance of entropy and a dilute solution. Yet, when J_p increases to $\geqslant 0.55\ k_BT$, $f_9(\phi)$ becomes non-monotonic and develops a local minimum at a high ϕ value, which agrees with our bulk simulation result that phase separation starts at $J_p = 0.55\ k_BT$ (see *SI Appendix*, Fig. S3). Thus, we take $f_9(\phi = 0.08) = -2.93\ k_BT$ as the free energy of a monomer in the bulk, which is equal to the value of $f_9(\phi)$ for the local minimum at high ϕ value when $J_p = 0.55\ k_BT$. Accordingly, $\phi = 0.08$ is taken as the bulk polymer concentration (ϕ^∞). Considering that at low concentrations, the enthalpic contribution to the Flory-Huggins free energy is small, $\phi^\infty = 0.08$ and $f_9(\phi^\infty) = -2.93\ k_BT$ are considered as constants for $J_p \leqslant 0.55\ k_BT$ (see Fig. 4*A*).

Free Energy Analysis of Surface Condensation. The three contributions to F are divided into two parts $F = F_{3D} + F_{2D}$, which are minimized separately like in the classical theory of wetting (18, 45, 46). Here F_{3D} is the free energy of forming a surface condensate of area A and density ϕ_0 from a uniform dilute polymer solution at concentration ϕ^∞. Specifically we have:

$$\frac{F_{3D}}{A} = \underbrace{\mu_0 D\phi_0}_{f_1} - \underbrace{J_p D\phi_0^2\frac{Z-2}{2}}_{f_2} + \underbrace{\int_D^\infty \left\{\frac{K}{2}(\nabla\phi)^2 + f_9(\phi)\phi - f_9(\phi^\infty)\phi\right\} dz}_{f_3} \quad [3]$$

$$\mu_0(\phi_0) = \frac{1-\phi_0}{\phi_0}\ln(1-\phi_0) - \frac{1}{L}\ln(\frac{L}{\phi_0}) - \frac{\ln(C_9^{surf})}{L} - f_9(\phi^\infty) \quad [4]$$

where D is the thickness of the surface condensate and $C_9^{surf} = 112,325$ is the number of conformations of a 9-monomer chain with a given starting site confined in the surface condensate (see *SI Appendix* for details). μ_0 in the term f_1 accounts for the loss of conformational and translational entropies per monomer when bulk polymers are confined to the surface condensate. f_2 is the enthalpic gain from the interaction between polymers in the surface condensate, and f_3 is the interfacial energy between the bulk polymer solution and the surface condensate.

While F_{3D} describes what happens above the membrane, F_{2D} is the free energy of membrane reorganization and forming tether-polymer interaction during surface condensation. Specifically we have:

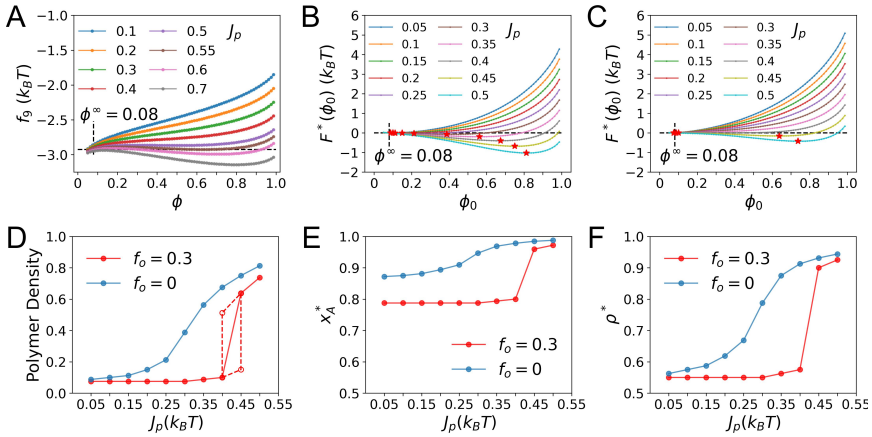

Fig. 4. Mean-field theory (MFT) for the effect of obstacles on surface condensation. (A) Free energy per monomer as a function of polymer density in the bulk solution, $f_9(\phi)$, at different $J_p(k_BT)$ calculated from the adapted Flory-Huggins theory. ϕ^∞ represents the bulk polymer density. (B) Minimized F as a function of polymer density in the surface condensate $F^*(\phi_0)$ at different $J_p(k_BT)$ at f_o = 0. Red stars label the positions where ϕ_0 minimizes $F^*(\phi_0)$ at different $J_p(k_BT)$, which have free energies lower than that of the dilute reference, leading to the formation of surface condensate. (C) Same as (B) but for f_o = 0.3. (D) Polymer densities; (E) lipid compositions; (F) tether concentrations of the surface condensates as a function of J_p at different obstacle fractions from the MFT calculations. In part (D) the left (right) empty red circle shows condensate density achieved in the calculation for J_p =0.4 (0.45) k_BT while the membrane configuration is fixed at that corresponds to the minimum of $F^*(\phi_0)$ in a normal calculation at J_p =0.45 (0.4) k_BT.

$$\frac{F_{2D}}{A} = f_{mem} - \underbrace{h_{teth} D \rho' \phi_0}_{f_{int}} + \underbrace{\frac{\rho'}{\rho}\left[\rho \ln(\rho) + (1-\rho)\ln(1-\rho)\right] - \lambda_\rho \rho'}_{f_{tether}} \quad [5]$$

$$f_{mem} = (1-f_o)\left[x_A \ln(x_A) + (1-x_A)\ln(1-x_A)\right] - \frac{Z'}{2} m^2 J_m (1-f_o)^2 - \lambda_m m \quad [6]$$

where m is the average magnetization of the condensate supporting membrane ($x_A = \frac{1+m}{2}$ is the fraction of lipid A), Z'=4 the coordination number of 2D square lattice, ρ the local tether density on lipid A, $\rho' = \rho(1-f_o)\frac{1+m}{2}$ the overall tether density on the local membrane (regardless of lipid type), h_{teth} the tether-polymer interaction strength, λ_ρ the chemical potential of tether and λ_m is one half of the difference between the chemical potentials of lipid A and B. f_{mem} and f_{tether} represent the contribution to the membrane free energy from lipids and tether, respectively. f_{int} is the interaction energy between tethers and polymers (see *Methods* for derivations). The parameter values used in the MFT calculations are summarized in *SI Appendix* Table S1.

With F_{3D} and F_{2D} defined, we then minimize them independently at fixed J_p and f_o values for each ϕ_0 to get $F^*_{3D}(\phi_0)$ and $F^*_{2D}(\phi_0)$. Therefore, the ϕ_0 that minimizes their sum $F^*(\phi_0) = F^*_{3D}(\phi_0) + F^*_{2D}(\phi_0)$, will be the density of the most stable surface polymer phase that forms at the given J_p and f_o (see *SI Appendix* and Fig. S10-11 for detailed discussions).

Minimization of $F(\phi_0)$. Without obstacles, the sum of the minimized $F^*_{2D}(\phi_0)$ (black curve of *SI Appendix* Fig. S11A) and $F^*_{3D}(\phi_0)$ (*SI Appendix* Fig. S10) is plotted as $F^*(\phi_0)$ in Fig. 4*B*. At low J_p values, $\phi_0 = \phi^\infty$ globally minimizes $F^*(\phi_0)$, indicating the absence of condensate formation. The situation of $\phi_0 < \phi^\infty$ is ignored considering that we have a polymer-attracting membrane. When J_p reach 0.1 k_BT, the local minimum of $F^*(\phi_0 = 0.1)$ (see the red star of the orange curve of Fig. 4*B*) becomes equal to $F^*(\phi_0 = \phi^\infty)$, which marks the onset of surface condensate formation. As J_p further increases, the global minimum of $F^*(\phi_0)$ gradually shift to the right (see red stars in Fig. 4*B*), reflecting the gradual increase of condensate density as J_p increases.

On the other hand, when f_o is increased to 0.3, $F^*_{2D}(\phi_0)$ and its derivative adopt different values (*SI Appendix* Fig. S11C). Consequently, the formation of surface condensate now starts at a higher J_p (0.4 k_BT), while featuring an increased density-to-J_p sensitivity, as manifested by the red star locations on the $F^*(\phi_0)$ curves in Fig. 4*C*. These observed differences in the condensate-to-J_p response, as well as the membrane composition-to-J_p response from adding obstacles are further summarized in Fig. 4*D* − *F*, which well recapitulate the corresponding observations from the simulation results (see Fig. 3*A* − *C*).

Physical Origin of the Obstacle Effects. The formation of surface condensation consists of several contributions: 1. the confinement of polymers from a dilute bulk solution into the surface condensate, which is unfavorable due to the loss of conformational and translational entropies of the confined polymers and the distortion of polymer concentration profile above the condensate; 2. formation of favorable polymer-polymer interactions in the surface condensate, where the polymer concentration is much higher than in the bulk solution; 3. concentration of specific lipids and tethers to the membrane beneath the condensate, which is unfavorable due to the loss of their translational entropies, especially above the critical temperature of the membrane; 4. formation of favorable tether-polymer interactions in the surface condensate. As such, surface condensate emerges only when the free energy gain of contributions 2 and 4 outweighs the loss of contributions 1 and 3. Therefore, to achieve a surface condensate of a given density ϕ_0 and certain membrane composition beneath it, which involve fixed free energies of contributions 1 and 4, the enthalpic gain of contribution 2 needs to overcome

the entropic loss of contribution 3. The presence of obstacles, which suppresses the concentration of membrane components in contribution 3 (as manifested in the simulation results and Eq. **5-6**), thus requires stronger polymer-polymer interactions to drive the formation of the surface condensate. This explains the right shift of the $\phi_0 - J_p$, $x_A - J_p$ and $\rho - J_p$ curves in Fig. 3$A - C$ and Fig. 4$D - F$, or the enhancement of selectivity towards J_p due to membrane obstacles for the formation of surface condensates.

While the above argument elucidates the delay of the onset of surface condensation to higher J_p, it doesn't guarantee the enhancement of sensitivity. In fact, now that it's harder to drive the membrane reorganization by increasing J_p in the presence of obstacles, one might expect it is even harder to drive fast condensate assembly. To gain further insights, we reason that instead of only focusing on how membrane respond to J_p, we should also inspect how the change of local membrane composition influences the condensate. Noticeably, in both the simulation and the MFT results, rapid condensate density increase and membrane reorganization are observed simultaneously (Fig. 3$A - C$ and Fig. 4$D - F$). Without obstacles, when the membrane is close to phase separation on its own, a modest enrichment of polymers in the surface condensate at low J_p could already effectively induce its reorganization. The membrane composition then responds to J_p or condensate density in a gradual manner (see blue curves in Fig. 3$A - C$ and Fig. 4$D - F$). By contrast, when the obstacles suppress lipid phase separation and scale down the effective lipid A-polymer affinity (see Eq. **5**), membrane reorganization only starts at a stronger J_p in a shallow manner and then is driven progressively higher (see red curves in Fig. 3$A - C$ and Fig. 4$D - F$). This leads to the realization that, with obstacles, condensate density and membrane composition become more sensitive to increasing J_p because they increase co-operatively. More specifically, it is exactly because it's harder to drive membrane reorganization due to obstacles that membrane composition responds to the increase of ϕ_0 (J_p) in a co-operative manner (Eq. **5**); i.e., the positive co-operativity between the coupled growth of condensate and local membrane domain ultimately leads to the sensitivity enhancement. This co-operative mechanism is further demonstrated by the red dashed lines in Fig. 3A, Fig. 4D and *SI Appendix* Fig. S12, where when J_p increases with frozen membrane configuration, condensate density responds much less, and vice versa.

Although the current study focuses on biomolecular system, the general principle that positive co-operativity between the coupled growth of two order parameters results in their faster increases applies to broader contexts. For example, the positive reciprocal effects between incidental news exposure via social media and political participation observed in communication studies reflects similar principles only with polymers and lipids replaced by social media exposure and political participation (47), respectively. Such a general principle is also demonstrated with an intuitive example of tennis practice (see *SI Appendix* and Fig. S13).

Concluding Remarks

In recent years, biological membranes have been recognized to play a regulatory role in the formation of biomolecular condensates (13–17), especially in the context of cell signaling. The general physical principles and molecular details that govern the robustness and sensitivity of such regulations, however, remain to be elucidated. For example, the recent study of Machta and co-workers (18), which played a major role in inspiring the current work, highlighted the potential significance of the membrane being close to its critical point (48); it was shown that, as the membrane approaches its critical point, the range of polymer interaction strength that leads to pre-wetting transition is greatly expanded. While this was an interesting observation, the insensitivity of the pre-wetting transition to the polymer interaction strength implies the lack of selectivity, making the mechanism less than ideal from a functional perspective. Moreover, as discussed here and in previous work (32, 33), realistic cellular membranes, which are rich in proteins, are unlikely to undergo macroscopic phase separation under ambient conditions.

Motivated by these considerations, in this work, we have studied the effect of protein obstacles on the coupled phase behaviors of biological membranes and surface biopolymers through GCMC simulations and a mean-field theory. We confirmed previous theoretical analysis (32, 33) that the presence of protein obstacles, especially immobile ones, suppresses phase separation of lipid membrane. Despite deviation from the critical point, the local membrane composition responds co-operatively to the condensation of surface polymers, which is in turn further promoted by the local enrichment of specific lipids; co-operativity in the coupled growth thus leads to enhanced sensitivity and selectivity of local membrane reorganization and pre-wetting transition to the properties of the polymer, represented by the interaction strength J_p in the current model. The key role of the obstacles in enhancing the sensitivity and selectivity is, in fact, to push the membrane away from conditions that strongly favor surface condensation, leaving opportunities for polymer properties to contribute.

The sensitivity and selectivity of the surface condensate to the polymer properties are functionally relevant; the polymer properties can be modified by variations in sequence, PTM state or local environmental variables such as pH. For example, a high level sensitivity of phase separation to these changes is essential for cells to produce digitized output in processing cytoplasmic or external signals through the mechanism of surface condensation (49). Previous studies have revealed that the assembly of condensates of the linker for activation of T cells (LAT) in the T cell receptor (TCR) signaling pathway responds nonlinearly to the phosphorylation states of its tyrosine sites; this feature was proposed to be correlated with the selectivity and sensitivity of TCR antigen discrimination (16, 17, 50, 51). The pH sensitivity of the condensate formation by the prion protein Sup35 was found to promote yeast cell fitness (52). Methylation of arginine sites was suggested to be an effective physiological regulator of fused in sarcoma (FUS) phase behavior (53). The condensation state of epidermal growth factor receptor (EGFR) and the adaptor protein Grb2 was revealed to be sensitive to their phosphorylation states, which could regulate downstream signal propagation to the mitogen-activated protein kinase (MAPK) pathway (54), and phosphorylation is also believed to regulate gephyrin mediated clustering of receptors in inhibitory synapses via charge-charge interaction driven phase separation (55). Our analyses suggest that membrane obstacles may contribute constructively to the sensitivity and selectivity of signal transduction processes mediated by phase separation.

Our observation that surface condensate formation helps promote local lipid segregation agrees with the recent experimental observation that aggregation of attached surface biomolecules can drive the formation of phase separated lipid domains in membranes at temperature well above its T_c (56–60) Although our model system is minimal and surface condensation in biology may feature much greater complexity, the results from the current study highlights that creating positive co-operativity between membrane and surface phase behaviors represents a general strategy for enhancing the sensitivity and selectivity of signal transductions. We anticipate that such predictions can be experimentally tested *in vitro* through reconstituting phase separating polymer systems in the presence of multi-component lipid membranes with tunable obstacle compositions (13).

The obstacles explored here are inert in that they do not feature any preferential interactions with any lipid or polymer. In reality, protein obstacles may have specific features that further modulate their impact on the coupled membrane/biopolymer phase behaviors. For example, obstacles with preferable interaction with selected lipids, which has been shown to eliminate membrane phase separation at a rather low density, could potentially be an effective way for implementing location selectivity in surface condensate formation (33, 34). In addition, obstacle's structural properties can also affect surface condensation formation. For example, the protruding parts of obstacles or protein-induced curvatures may introduce surface roughness that places a length-scale threshold on proteins that are capable of conformal coating and the subsequent condensation (61–63). Finally, how non-equilibrium processes, which are prevalent in signal transductions, contribute to the coupled phase behaviors of complex membrane and surface biopolymers and, therefore, sensitivity and selectivity of cellular responses is a fascinating topic for future explorations.

Methods

MC Simulations of the Membrane. The lipid membrane is modeled by fixed composition 2D Ising model depicted in the main text with Hamiltonian given in Eq. **1** repeated below.

$$H_{Ising} = -J_m \sum_{i,j \in NN} S_i S_j$$

Simulation for each membrane condition (J_m and the concentration of immobile/floating obstacles) consists of 10^6 MC sweeps through all lipid and mobile obstacle sites. MC moves are accepted with the Metropolis probability $p_{accept} = min\left\{e^{-\beta(H_f - H_i)}, 1)\right\}$, where H_i/H_f is the energy of the system before and after the proposed move and $\beta = \frac{1}{k_B T}$. $P(x_A)$ of the membrane is averaged over configurations generated every 100 MC sweeps.

GCMC Simulations of the Polymer-Membrane System. The coupled polymer-membrane system is modeled as depicted in the main text, whose Hamiltonian is given by Eq. **7** below.

$$H = H_{Ising} \underbrace{- J_p \sum_{i,j \in NN} \sigma_i \sigma_j + \mu N_p}_{H_{polymer}} \underbrace{- J_t \sum_{i \in tethers} \sigma_i}_{H_{int}} \quad [7]$$

Here, $\sigma_i = 1/0$ if a lattice site i above the Ising membrane is occupied/unoccupied by a monomer. NN interaction between two connected monomers from the same polymer chain is not counted. A lattice site can not be occupied by more than one monomer. $\mu = -19 k_B T$ is the chemical potential of a single 9-monomer chain and N_p is the total number of polymer chains in the simulation box. J_t is the interaction energy between polymers and tethers. $i \in tethers$ include all lattice site i that is occupied a tether, thus a polymer-tether interaction forms when a monomer occupies the same lattice site as a tether. A tether must be attached to a lipid-A membrane site and cannot overlap with other tethers. The simulation box contains 40×40×30 (xyz) lattice sites and has PBC in the $x-y$ plane with non-penetrable boundaries in the z direction.

In the simulation of the coupled system, each MC sweep is divided into two sequential steps. The first step contains $N_{mc} = \frac{5}{3}(N_p + N_t)$ MC moves, where N_p is the number of polymer chains in the system before the start of the MC sweep, and N_t is the total number of tethers in the system (fixed at initialization). 40% of the N_{mc} MC moves are assigned to the addition and deletion moves of polymers equally, while the remaining 60% are assigned to polymer moves and tether moves in the ratio of $N_p : N_t$. All N_{mc} moves are then carried out in a randomized sequence. The second step of the MC sweep is the membrane simulation, which sweeps through all lipid and mobile obstacle sites like described in the previous section, except that tether attached lipid-A sites are not allowed to move. Surface condensate is identified as the $5 \times 5 \times 5$ (or sometimes $10 \times 10 \times 5$) (xyz) surface region of the highest monomer occupancy of the system. The properties of the system is averaged over configurations generated every 100 MC sweeps in a typical simulation of 10^6 MC sweeps.

In a polymer addition move, a 9-monomer chain (9-mer) is selected randomly from a pre-built pool that contains all possible 9-mer configurations. This selected 9-mer is then proposed to be added to the simulation box with its center to be placed at a randomly selected lattice site. The probability of acceptance is $p^{accept}_{N_p \to N_p+1} = min\left\{e^{-\beta(\triangle U - \mu)} \frac{V N_{conf}}{N_p+1}, 1)\right\}$, where $\triangle U$ is the energy change of the system by the proposed move, N_p the number of 9-mers in the system before the move, V the system size and N_{conf} the number of all possible 9-mer configurations (64).

In a polymer deletion move, a randomly selected 9-mer from the current simulation box is proposed to be deleted with $p^{accept}_{N_p \to N_p-1} = min\left\{e^{-\beta(\triangle U + \mu)} \frac{N_p}{V N_{conf}}, 1)\right\}$. Addition or deletion of a 9-mer is immediately rejected if it leads to monomer overlap, change of the number of inter-chain interactions or polymer-tether interactions in the system. Special care is taken to treat the boundaries in the z direction in terms of the values of N_{conf}.

In a tether move, a randomly selected tether is proposed to translate one lattice site on the $x-y$ plane with the Metropolis acceptance ratio. A tether can only be moved to neighboring lipid-A sites.

In a polymer move, for the majority of the time, a randomly selected 9-mer is proposed to do reptation move, in which a bond is removed from one end of the chain and glued to the other end at a random direction. A reptation move is accepted with the Metropolis probability while no monomer overlap is allowed. With a probability of $\frac{2.2}{N_p}$ we propose cluster move of the selected 9-mer. A cluster is identified as the collection of all polymers connected to the selected 9-mer

directly or indirectly through monomer-monomer NN contact. The cluster is proposed to move one lattice site at a random direction with the Metropolis acceptance ratio, while moves that result in the growth of the cluster are rejected to satisfy detailed balance (35).

The effectiveness of such simulation scheme in exchanging polymers between the dense condensate and the dilute solution is further demonstrated in *SI Appendix* Fig. S14

Adapted Flory-Huggins Theory. In the classical Flory-Huggins theory, the number of independent configurations for arranging N polymers of length L in M lattice sites is given by:

$$\Omega = \frac{M!}{N!(M-NL)!}(\frac{Z-1}{M})^{N(L-1)}$$

where Z is the coordination number of the lattice. In this work, we replace the part for counting conformational entropy $(z-1)^{N(L-1)}$ with C_9^N to avoid intra-chain overlap. Combined with the mean field energy $U = -NL\frac{(Z-2)NL}{2M}J_p$ we have the free energy per monomer given in Eq. **2**:

$$\begin{aligned} f_9 &= \frac{U-TS}{M\phi k_B T} \\ &= \frac{U-k_BT\ln(\Omega)}{M\phi k_B T} \\ &= -\phi\frac{Z-2}{2}J_p + \frac{(1-\phi)}{\phi}\ln(1-\phi) - \frac{1}{L}\ln(\frac{L}{\phi}) - \frac{1}{L}\ln(C_9) \end{aligned}$$

where $\phi = NL/M$ is the polymer density.

Minimization of F_{3D} in Mean-field Theory. At given ϕ_0 and J_p (f_1 and f_2 are fixed), the minimization of F_{3D} is just the minimization of the integration in the term f_3 over the polymer density profile $\phi(z)$ above the surface condensate. To satisfy $\frac{\delta f_3(\phi(z))}{\delta\phi(z)} = 0$ we have:

$$\begin{aligned} K\frac{\partial^2\phi}{\partial z^2} &= \frac{\partial[f_9(\phi)\phi - f_9(\phi^\infty)\phi]}{\partial\phi} \\ \frac{K}{2}\frac{\partial}{\partial z}(\frac{\partial\phi}{\partial z})^2 &= \frac{\partial[f_9(\phi)\phi - f_9(\phi^\infty)\phi]}{\partial z} \\ \frac{K}{2}\int_D^\infty dz\frac{\partial}{\partial z}(\frac{\partial\phi}{\partial z})^2 &= \int_D^\infty dz\frac{\partial[f_9(\phi)\phi - f_9(\phi^\infty)\phi]}{\partial z} \\ \frac{K}{2}\left(\frac{\partial\phi}{\partial z}\right)^2_{z=D} &= [f_9(\phi_0)\phi_0 - f_9(\phi^\infty)\phi_0] \end{aligned} \quad [8]$$

Substituting Eq. **8** into f_3 we have:

$$\begin{aligned} f_3 &= \int_D^\infty K\left(\frac{\partial\phi}{\partial z}\right)^2 dz \\ &= \int_{\phi_0}^{\phi^\infty} K\left(\frac{\partial\phi}{\partial z}\right) d\phi \\ &= \int_{\phi_0}^{\phi^\infty=0.08} d\phi\left(-\sqrt{2K[f_9(\phi)\phi - f_9(\phi^\infty)\phi]}\right) \end{aligned} \quad [9]$$

Eq. **9** (minimized f_3 for a given ϕ_0 and J_p) can then be easily evaluated numerically.

Lipid membrane Free Energy. The lipid free energy is obtained through a mean-field treatment. Specifically, the energy and entropy of a membrane of area A (lattice sites), with obstacle fraction f_o and average magnetization m is given by

$$E_{mem} = -J_m\frac{Z'A''}{2}(P_{aa} - P_{ab}) \quad [10]$$

$$S_{mem} = k\ln(\begin{pmatrix} A'' \\ A''\frac{1+m}{2} \end{pmatrix}) \quad [11]$$

$$f_{mem} = \frac{1}{A}(E_{mem} - TS_{mem}) - \lambda_m m \quad [12]$$

where $A'' = A(1-f_o)$ is the number of total lipids, $P_{aa} = (\frac{1+m}{2})^2 + (\frac{1-m}{2})^2$ and $P_{ab} = \frac{1-m^2}{2}$ are the probability of a bond between like and unlike lipid pairs. With Stirling approximation, it can be shown that f_{mem} of Eq. **12** is equal to f_{mem} in Eq. **6**. f_{mem} derived here leads to the same critical point $J_m^c = \frac{k_BT}{z'=4}$ for 2D Ising model as usually seen in the MFT (see *SI Appendix* Fig. S15) (65).

Tether Free Energy. The entropy of having a fraction ρ of the A' lipid sites to be attached to a tether (non-interacting) is:

$$S_\rho = k\ln(\begin{pmatrix} A' \\ A'\rho \end{pmatrix}) \quad [13]$$

where $A' = A(1-f_o)\frac{1+m}{2}$ is the number of up spin sites within area A. Then the free energy contribution per unit area from tether arrangements is:

$$f_{tether} = \frac{-TS_\rho}{A} - \lambda_\rho\rho' \quad [14]$$

With Stirling approximation, it can be shown that f_{tether} of Eq. **14** is equal to f_{tether} in Eq. **5**.

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