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Biophysical Chemistry 104 (2003) 217–227

Biophysical Chemistry

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Self organization of membrane proteins via dimerization

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Received 29 October 2002; received in revised form 13 November 2002; accepted 13 November 2002

Abstract

Protein–protein dimerization is ubiquitous in biology, but its role in self-organization remains unexplored. Here we use Monte Carlo simulations to demonstrate that under diffusion-limited conditions, reversible dimerization alone can cause membrane proteins to cluster into oligomer-like structures. When multiple distinct protein species are able to form dimers, then heterodimerization and homodimerization can organize proteins into structured clusters that can affect cellular physiology. As an example, we demonstrate how receptor dimerization could provide a physical mechanism for regulating information flow by controlling receptor–receptor cross talk. These results are physically realistic for some membrane proteins, including members of the G-protein coupled receptor family, and may provide a physiological reason as to why many proteins dimerize.

Keywords: Dimerization; G-protein coupled receptor; Monte Carlo; Diffusion-limited aggregation; Oligomerization

1. Introduction

Many proteins form hetero- or homodimers, but why? For example, a large number of G-protein coupled receptors (GPCRs) are able to form homo- and heterodimers (reviewed in [1,2]), but the reason for this dimerization remains elusive. In most cases, dimerization of GPCRs does not directly correlate with the receptor's signaling state, as some receptors form dimers in the active state while others form dimers in the inactive state. It has been suggested that dimerization influences receptor cross-talk or desensitization, but the mechanism for this interaction is not currently known [3,4]. Similarly, receptor dimerization not directly related to signaling is common in other signal transduction pathways. For example, recent findings have suggested that dimerization of the epidermal growth factor receptor is independent and separable from receptor signaling [5]. Similarly, the bacterial receptor Tar [6], human nerve growth factor receptor [7], and the bacterial and plant Photosystem II proteins [8] are all able to reversibly form dimers in the membrane, but the reasons for these interactions are largely unknown.

Here we propose a diffusion-limited mechanism by which receptor dimerization can drive the formation of oligomer-like clusters. We further

Abbreviations: GPCR: G-protein coupled receptor.

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PII: S0301-4622(02)00369-1

demonstrate that such clusters could affect physiological processes such as cross-talk.

It is important to distinguish the one-to-one process of dimerization from non-specific oligomerization. GPCRs, like other membrane bound receptors, contain residues that mediate the specific protein–protein binding events that permit dimerization [9]. As a result of these specific interface sites, when a dimer is formed between two receptors, the binding face is covered, thereby disallowing any additional bonds to form. Therefore, receptors with only one specific binding site can only form dimers and cannot form stable larger structures such as trimers or oligomers. In contrast, non-specific oligomerization or aggregation takes place when proteins have many, non-specific binding sites, such as when a protein is denatured or misfolded. This process of non-specific oligomerization can be adequately simulated using condensed matter models, such as hard spheres interacting via a Leonard–Jones or square well potential [10]. Similarly, non-specific lattice-gas models of dimer–dimer interactions have also been proposed [11]. Specific dimerization, in contrast, cannot be described this way because once a dimer is formed, the effective attractive potential of the dimer for other particles drops to zero.

Therefore, although dimerization is common in biology, new models are needed to understand the physiological role of dimerization. For example, how does dimerization affect protein localization in the membrane? How does this dimerization-induced organization in turn affect cellular processes, such as signal transduction? In this work we use computer simulations to address both these questions.

We hypothesize that dimerization could have long-range ordering effects via a partner switching mechanism shown in Fig. 1. In this view, each protein competes to bind with its neighbors before they move too far apart to interact. If the partner switching is fast relative to the diffusion rate, then the proteins can effectively share a single bond between multiple proteins and in doing so form clusters of proteins that extend beyond a dimer pair. By modifying the localization of proteins in the membrane, dimerization could provide an elegantly simple way to control access of signaling elements to each other, thereby affecting signaling related processes such as receptor cross-talk.

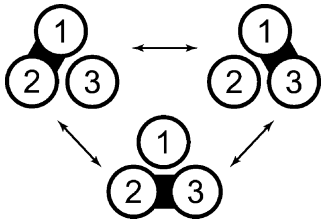


Fig. 1. Dimerization can lead to the formation of trimers via diffusion-limited partner switching. This same mechanism can be extended to form larger oligomers when more monomers are present.

The findings of this study suggest that GPCRs can exhibit dimerization-induced oligomerization like that shown in Fig. 1, and that such organization can in turn affect receptor signaling. This finding is in agreement with a number of unexplained experimental results relating to receptor cross talk and GPCR cluster size.

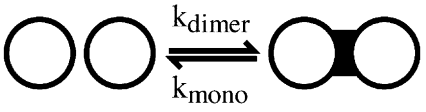
2. Methods

In developing a model for protein dimerization in the membrane, we strove to keep the model as simple as possible such that the effects of dimerization alone could be studied. In this spirit, we approximated proteins as hard disks with a single binding site. The processes that govern the dimerization rate of two adjacent proteins, such as protein rotation within the membrane and the reaction rate of two aligned proteins, were collapsed into a single, intrinsic dimerization rate constant, k_{dimer}. As a result, monomers were allowed to bind to any monomer within a specified radius at a rate controlled by k_{dimer}. In forming a dimer, however, the proteins have their binding interfaces fixed pointing toward each other and no further protein binding was allowed. The processes leading to the dissociation of a dimer into two monomers were similarly collapsed into a single rate constant, k_{mono}. Upon monomerization, the binding face is released, thereby allowing the binding interface to move freely within the

membrane once more. The details of the simulation and data analysis are described below.

2.1. Monte Carlo simulations

The process of membrane protein dimerization was modeled by simulating the diffusion and reaction events of discrete proteins on a two-dimensional surface. Diffusion was governed by the diffusion coefficient and dimerization and monomerization were described by two reaction rate constants, k_{dimer} and k_{mono} in the reaction:



(1)

Note that k_{dimer} is an intrinsic rate constant, meaning that it describes the rate at which binding takes place after diffusion has brought the proteins within reaction range.

Simulations were run on a 700×700 triangular lattice with periodic boundary conditions and a lattice spacing corresponding to 0.5 nm. Proteins were assumed to occupy hexagons with a diameter of 10 lattice spacings, which corresponds to a protein diameter of 5 nm and is approximately equal to the diameter of a single G-protein coupled receptor. By using a fine grid, we were able to approximate diffusion on a continuous surface. Smaller lattice spacings were tested and found to have no effect on the overall clustering behavior.

During the simulation, proteins were picked at random to react and move. If the chosen action was a dimerization event, then the protein was first tested to see if it was a monomer. If the chosen protein was a monomer, then a random neighboring monomer within an interaction radius of 5 lattice spacings (2.5 nm) was chosen as a binding partner. Dimerization reactions between two adjacent monomers were allowed with a probability, P_{dimer}. Dimers were allowed to monomerize with a probability P_{mono}. With a discrete time model like the one used here, the dimerization and monomerization reactions can be modeled as Poisson processes. As a result, the probabilities of dimerization and monomerization can be derived from the bulk reaction rates k_{dimer} and k_{mono} using a Poisson distribution. With sufficiently small time steps (Δt), this relationship simplifies such that the probability of a reaction is nearly proportional to the reaction rate [12], as is shown in Eq. 2 below

$$P_{\text{mono}} = 1 - e^{-k_{\text{mono}}\Delta t} \approx k_{\text{mono}}\Delta t \tag{2}$$

$$P_{\text{dimer}} = 1 - e^{-k_{\text{dimer}}\Delta t} \approx k_{\text{dimer}}\Delta t$$

In a diffusion event, a protein was allowed to move a single lattice spacing in a random direction. If the site was occupied, then the move was rejected and not repeated. If the protein was part of a dimer, then additional constraints were placed on its movement. A single protein within a dimer was allowed to pivot around its partner or move toward its partner, assuming the separation distance between the two proteins was greater than or equal to the hard sphere diameter of the protein. If a protein within a dimer pair attempts to move away from or overlap with its partner, then an attempt was made to move both proteins in lock step. If the new positions of either protein were blocked, then the move was rejected and not repeated. Otherwise, both proteins were moved. As a result of these diffusion rules, proteins move with approximately the same diffusion coefficient independent of their dimerization state. This property is consistent with theoretical findings that show that diffusion is only a weak function of particle radius [13].

The probability of a diffusion event, P_{move}, was calculated using the translational diffusion coefficient D_t of the protein. For a single particle exhibiting Brownian diffusion on a triangular lattice, the probability of a particle moving at least one lattice spacing, l, in one iteration time step, Δt, can be expressed as

$$P_{\text{move}} = 1 - \exp\left(\frac{-6\Delta t D_t}{l^2}\right) \approx \frac{6\Delta t D_t}{l^2} \tag{3}$$

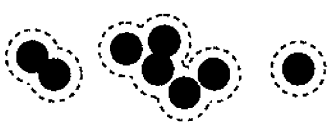
Thus, at small time step values, the probability of a move is nearly proportional to the diffusion

coefficient of the protein in the membrane [12,14]. In all simulations, the value of Δt was chosen such that the probability of the most likely event was 10%.

To apply our simulation results to GPCRs, we determined values for D_t, k_{dimer}, and k_{mono} specific to this receptor family. For GPCRs, values for D_t range from 10^{-8} to 10^{-11} cm^2/s [13,15]. The dimerization rate constant, k_{dimer}, for two adjacent GPCRs has not been measured. To estimate a maximum value of k_{dimer}, we first assumed that proteins would dimerize with 100% probability once they were properly aligned. We then simulated the Brownian rotation of two adjacent receptors, with the probability of a rotation event calculated from the observed GPCR rotational diffusion coefficient of 2.7×10^5 s^{-1} [13]. Starting at a random initial configuration, simulations were carried out to determine the mean time required for two receptors to align to within 60° of the protein–protein binding site corresponding to a proper alignment configuration. Under these conditions, we calculate a maximum k_{dimer} value for GPCRs to be on the order of 10^5 s^{-1}. Monomerization, in contrast, is not affected by the rotational diffusion of the proteins. As such values of k_{mono} are expected to vary widely depending on the identity and state of the receptor.

2.2. Analysis of simulation output

Statistics for average cluster size and average shortest separation distance between particles were gathered to assess the effects of changing the simulation environment. Average cluster size was measured by counting the total number of proteins that are within the interaction radius of at least one member of the same cluster. As a result, clusters generally include a mixture of both monomers and dimers. Examples of clusters of size 2, 5 and 1 are shown below



(4)

Separation distance was measured by counting the number of grid spacings between a particle and its nearest neighbor of a specific type.

Before statistics were taken, all simulations were allowed to pre-equilibrate. For each condition at least 500 distinct measurements were made. Parameter regime simulations were run with 1000 particles corresponding to a surface coverage of 18% and reached equilibrium within 10 ms of simulated time. Runs with two protein species were performed with 300 of each species and $k_{\text{mono}}=4.6$ and $k_{\text{dimer}}=46$, placing the ensemble in the oligomer regime. Average separation distance was measured for 10 000 independent runs. Simulations were written in C^{++} using Metrowerks CodeWarrior and were run on a cluster of Apple PowerPC G4 machines. Source code is available from the authors upon request.

3. Results

The results of the simulations follow from simplest to more complicated. Under the simplest conditions, dimerization of only one species was simulated under a wide range of k_{mono} and k_{dimer} values to see if an oligomer regime could be detected. Next, simulations were performed with the addition of a second, inert species to better simulate conditions in the real cell membrane. Finally, we explore the effects of homo- and heterodimerization with two receptor species. Together these results give us a more complete picture of the ability of dimerization to organize proteins on the cell membrane.

3.1. Dimerization alone can cause clustering

We first consider the case of one homodimerizing protein that is allowed to react and diffuse in two dimensions. Under these conditions we observe three qualitatively different regimes that are shown schematically in Fig. 2a, which is derived from our simulation results in Fig. 2b. Depending on the binding kinetics relative to the diffusion rate, the ensemble behaves as a two-dimensional gas of monomers, a two-dimensional gas of dimers, or as a mixture of diffusion-limited

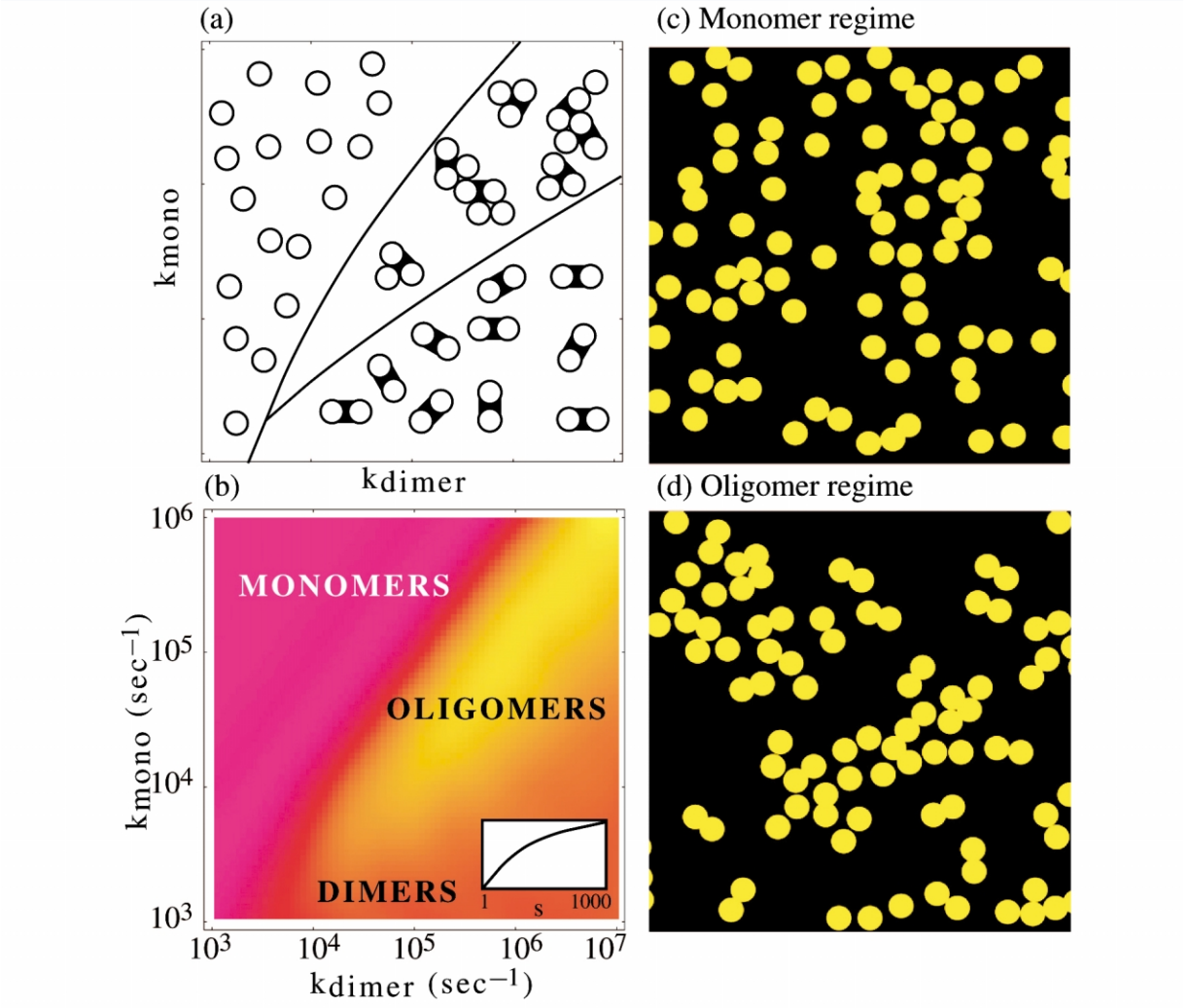


Fig. 2. (a) Schematic behavior of a dimeric species to indicate three regimes: a two-dimensional monomer gas, a two-dimensional dimer gas, and a diffusion-limited oligomer regime. (b) Simulation results that show how the cluster size varies with k_{mono} and k_{dimer}. Simulations performed with a diffusion coefficient of 10^{-9} cm^2/s. The labels on the diagram denote the three observed regimes. Cluster size is indicated by color and varies from 4.2 to 1.9 (4.2 1.9) particles per cluster. Inset figure plots the average cluster size as a function of the kinetic rate of binding, σ, at a constant equilibrium K_d value of 0.1. (c) A sample image of the protein organization in the monomer gas regime. (d) A sample image of the protein organization in the oligomer regime. Note that the proteins are more clustered in the oligomer regime than they are in the monomer regime, as is reflected quantitatively in b.

oligomers. In the monomer gas regime, the ratio k_{dimer}/k_{mono} is low, driving the equilibrium toward a homogeneous mixture of monomers. In the dimer gas regime, the ratio k_{dimer}/k_{mono} is high, permitting the proteins to form stable dimers but lacking any long-range order.

Between the two extremes of a monomer gas and a dimer gas is an intermediate state that orders

proteins into oligomers via the partner switching mechanism described in Fig. 1. By oligomer, we mean a tightly packed cluster of receptors made up of dimers and monomers as is described in the methods section. Our simulation results shown in Fig. 2b demonstrate that this ordered oligomer regime emerges from systems of many particles when only dimerization, monomerization, and diffusion are present. Movies of this interaction can be viewed on the web (www.mit.edu/~pwoolf/dimerization.html.) Oligomer size is maximized when the dimerization rate is approximately ten times the monomerization rate, and when both rates are fast relative to the diffusion rate. From inspection of Fig. 2b, the minimum dimerization and monomerization rate constants required to observe the oligomer regime are approximately $k_{\text{dimer}}=10^5\ \text{s}^{-1}$ and $k_{\text{mono}}=10^4\ \text{s}^{-1}$, with larger constants resulting in further increases in oligomer size. Fig. 2c–d show examples of particle organization in the monomer and oligomer regimes, respectively, demonstrating that dimerization induced clustering causes a qualitatively different organization.

Note that the simulations were performed using $D_t=10^{-9}\ \text{cm}^2\ \text{s}^{-1}$, which represents an upper bound of this value. Lower values of D_t, which are also physiologically reasonable, rescale the data in Fig. 2b to favor cluster formation at lower values of k_{dimer} and k_{mono}. Thus, at $D_t=10^{-10}\ \text{cm}^2\ \text{s}^{-1}$, the oligomer regime appears at $k_{\text{dimer}}=10^4\ \text{s}^{-1}$ and $k_{\text{mono}}=10^3\ \text{s}^{-1}$ (data not shown).

The dimerization and monomerization rate constants for GPCRs have not been directly measured, but our estimates suggest that diffusion-limited oligomers can exist in the cell. As discussed in the methods section, the dimerization rate constant can be estimated based on the rotational diffusion coefficient. For a GPCR, k_{dimer} is predicted to be on the order of $10^5\ \text{s}^{-1}$, thereby allowing the system to access the oligomer regime. Increased mobility of the receptor–receptor binding surface or local structures in the lipid bilayer could also act to increase k_{dimer} and in doing so further favor oligomerization [16]. The monomerization rate constant is specific to the receptor species and its activation state, likely varying from nearly zero to much larger values and thereby spanning the monomer, dimer, and oligomer regimes.

Note that the different regimes described in Fig. 2b are primarily due to the kinetics of receptor dimerization and not the equilibrium values. States of constant equilibrium dissociation constant, K_d, can be described in Fig. 2b by drawing a straight line from the origin. Along these lines of constant K_d, the equilibrium ratio of $k_{\text{mono}}/k_{\text{dimer}}$ is constant while the kinetics described by k_{mono} and k_{dimer} can change, thereby decoupling kinetic and equilibrium effects in determining average cluster size. As the inset in Fig. 2b shows, scaling both k_{mono} and k_{dimer} by a constant factor, σ, at a constant K_d value of 0.1, results in an increase in the average oligomer size due to kinetic effects alone.

The observation that the kinetics of dimerization affect oligomer size makes intuitive sense, because in the limit of infinite σ values, the dimer bond becomes a field effect and not a discrete effect. In this limit, dimerization behaves like a modified square well potential, except that proximity to new binding partners changes the effective depth of the potential well. At the other extreme of σ values near zero, dimerization becomes an irreversible process where no bond sharing is possible. In this limit, proteins interact only as hard disks, resulting in a gas like state with a small average cluster size. Therefore, the kinetics of protein–protein dimerization are expected to play a central role in determining the structure of protein complexes within the membrane.

3.2. Effects of protein density on oligomer size

The effect of dimerization-induced oligomerization increases with increased protein density. Proteins in the membrane can be divided into two classes: (1) active proteins that can form dimers and (2) inactive proteins that cannot form dimers. In simulations, increasing the active protein concentration from 18 to 37% surface coverage results in a disproportionately large increase in average oligomer size when proteins are in the oligomer regime. Similarly, adding inert proteins up to a 37% surface coverage while maintaining an 18% coverage of active protein causes the average oligomer size to increase by approximately 15%

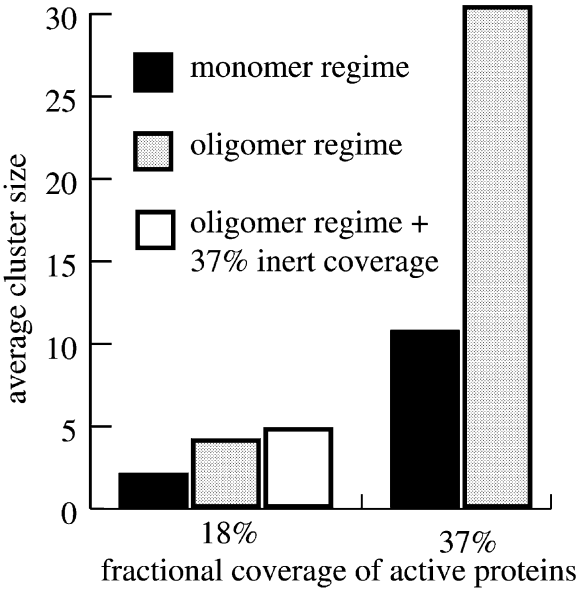


Fig. 3. Effects of increasing active and inert protein density on the average cluster size. Cluster size always increases with protein density due to simple crowding; however, in the oligomer regime the cluster size increases more quickly due to a reduction in the apparent diffusion coefficient at high protein density.

(Fig. 3). In the typical eukaryotic cell, proteins occupy between 20 and 50% of the membrane surface area [17]; therefore the protein densities tested in the simulations are physiologically realistic. Both increased active protein and inert protein density effectively reduce the observed diffusion rate of proteins within the membrane, driving the system into a more diffusion-limited regime that favors oligomerization. In Fig. 2b, this decrease in diffusion coefficient corresponds to shifting the results to the upper right, thereby favoring an increase in oligomerization.

Physiologically, this finding implies that dimerization-induced oligomerization plays an increasingly significant role when the local protein density is high. Although a receptor may be expressed at low densities on the cell surface as a whole, the receptor could be constrained to high density domains due to corralling or association with membrane lipid islands [16–19], thereby favoring oligomerization. In these high density domains, dimerization-induced oligomerization would be favored, suggesting a physiological mechanism for regulating oligomerization.

3.3. Dimerization influences receptor cross-talk

Next, receptor cross-talk was examined by simulating the interaction between two different protein species. For GPCRs, one type of cross-talk takes place when two receptors interact with a common G-protein. This kind of cross-talk can be beneficial because it allows many receptors to activate a common signal transduction pathway and thereby ensures that the signal will be transmitted; however, in other cases cross-talk could be harmful because it prevents the cell from discriminating between distinct pieces of information about the environment. Therefore, a mechanism for dynamically regulating receptor cross-talk would be of benefit to the cell.

The simulations predict that cross-talk at the receptor level depends on how the receptors associate with each other. We examined the interaction of two receptor species, A and B, under three different association rules: no dimerization (A and B are inert); homodimerization (A binds with A; B binds with B); and heterodimerization (A binds with B). Examples of each of these cases have been reported for GPCRs [3,4,20]. As a measure of receptor cross-talk we calculated the minimum distance separating two different species of receptors on the assumption that receptors spaced more closely would have more cross-talk via secondary messengers. Although protein–protein separation distance seems to be an obvious candidate for regulating cross-talk, here we provide a novel mechanism for regulating this distance via dimerization.

In the oligomer and dimer gas regimes, changing the receptor–receptor association rules affected receptor cross-talk (Fig. 4). No dimerization (Fig. 4a) led to a well-mixed system, resulting in an intermediate minimum separation distance between A and B. Heterodimerization (Fig. 4b) coupled dissimilar receptor species, resulting in a short separation distance between A and B and presumably increased cross-talk. In contrast, homodimerization (Fig. 4c) caused like receptor species to separate themselves into distinct islands, increasing

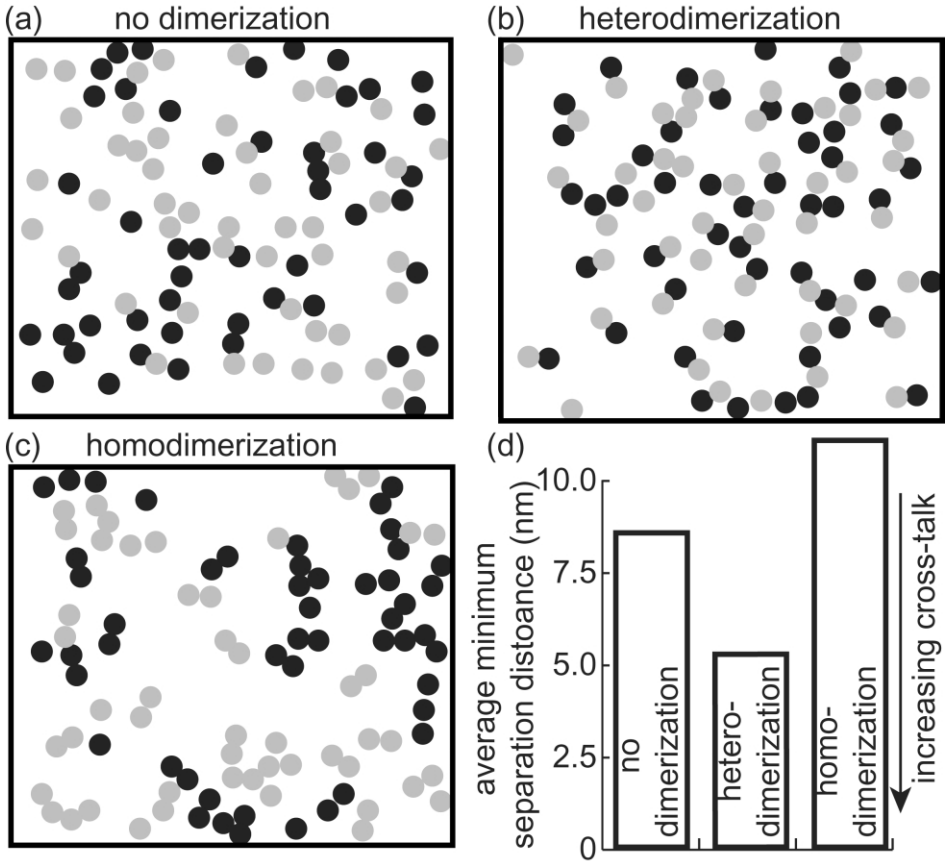


Fig. 4. Snapshots of the positions of two protein species in grey and black under the following protein–protein association rules: (a) No interaction between proteins; (b) Heterodimerization; (c) Homodimerization. (d) Average minimum separation distance between two dissimilar neighbors quantifies what can be observed visually in frames a, b and c, implying that heterodimerization enhances cross-talk while homodimerization represses cross-talk.

the separation distance between A and B and reducing cross-talk. Movies of this process can be found on the web (www.mit.edu/~pwoolf/dimer.html). Treatments that alter the receptor–receptor association rules, such as the presence of ligands that induce or inhibit dimerization, could in turn affect receptor cross-talk and any ensuing physiological responses.

The effects of receptor–receptor association rules on cross-talk are most pronounced in the oligomer regime. For example, when receptors associate as homodimers they form extended homogeneous islands in the oligomer regime, but only form stable pairs in the dimer gas regime. As such, homodimerization in the oligomer regime isolates dissimilar receptors more effectively than homodimerization in the dimer gas regime, presumably leading to reduced cross-talk. When receptors associate as heterodimers, cross-talk becomes sensitive to the concentration of each receptor species. In the dimer gas regime, any deviation from a 1:1 concentration ratio leaves uncoupled receptors, resulting in poor mixing and less cross-talk. In contrast, receptors in the oligomer regime use the partner switching mechanism (Fig. 1) which is less sensitive to the concentration ratio, resulting in better mixing over a wider range of conditions and more cross-talk. These results

may explain why evolution has favored dimerization in so many receptor systems and suggest that these receptor systems should operate in the oligomer regime for improved control of cross-talk.

4. Discussion

4.1. Dimerization of a single species

The results in Fig. 2 describe a situation in which the dimerization of a single species can organize receptors into clusters. This finding is physiologically relevant because GPCRs likely have the ability to exist in the monomer gas, dimer gas, or oligomer regimes depending on their dimerization kinetics and diffusion coefficients. Dimerization kinetics could be altered by the presence or absence of ligands, which has been experimentally demonstrated to regulate the dimerization state of some GPCRs [1]. Similarly, the diffusion coefficient of a receptor can vary from 10^{-9} to 10^{-11} depending on the receptor identity and local environment surrounding the receptor. Thus, at lower diffusion coefficient values, dimerization induced clustering is expected to be significant at lower k_{mono} and k_{dimer} values.

The model prediction that dimerization can cause the formation of oligomers is consistent with hereto unexplained experimental data for GPCRs. For example, the D_3 dopamine, δ-opioid, μ-opioid, and muscarinic M_2 receptors have all been shown to form not only dimers, but also trimers and tetramers [4,21,22]. Structural studies of these receptors have shown that these receptors have only one protein–protein binding site [23], and as such cannot form oligomers via multivalent interactions. However, these monovalent proteins could still oligomerize via the partner switching mechanism proposed in Fig. 1. Although it is possible that the trimers and tetramers observed in these experiments are artifacts of the experimental technique, the findings are consistent across many experimental systems and as of this time represent the only way to distinguish protein dimers from larger oligomeric structures. Future experimental techniques, such as cryo-AFM, may be able to provide sufficient resolution to visualize the locations of an ensemble of receptors on the cell membrane, and thereby observe the formation of oligomers [24]. However, these methods are still under development and as such are not widely available for such studies.

Oligomerization via dimerization is not only applicable to membrane proteins but also can be generalized to other non-biological systems. For example, heteroflocculation in colloidal suspensions is used industrially to control material properties [25]. In some cases this process may employ similar partner switching mechanisms that could be exploited to control the material properties of the suspension. Similarly, these results could be extended to apply to polyvalent particles, assuming that bond formation is reversible and that the rates of bond formation and destruction are fast relative to the diffusion rate.

4.2. Dimerization of multiple species

When multiple species are able to interact via hetero- and homodimers, more complex regulatory processes become possible. For example, the data in Fig. 4 demonstrate that changes in the dimerization rules of a single species can affect the global organization of the whole system. This same process could be easily extended to describe the interactions between more than two species, yielding more and more sophisticated behaviors in response to changes in dimerization states.

As one example of dimerization and crosstalk in an experimental system, receptor cross-talk has been directly measured between the α_{2b}-adrenergic, M_4 muscarinic, and δ-opiate receptors [26]. Because all three receptors act via a common secondary messenger, the G-protein G_i, it is expected that activation of one receptor species would deplete the pool of G_i available to the other receptors and in doing so would attenuate their signaling ability. Puzzlingly, this depletion induced cross-talk is not observed and each receptor species retains its signaling ability independent of the activity of the other receptor species [26].

A novel explanation of this lack of cross-talk is offered by our simulation results. According to the results presented in Fig. 4, cross-talk between receptors can be reduced if the receptors form homogeneous islands, each of which could control

a local pool of G-proteins. The simulations predict that this form of spatial separation could be achieved by receptor homodimerization in the oligomer regime similar to that shown in Fig. 4c. This prediction is corroborated by the finding that all three receptor species are reported to form homodimers [4,23,27–30], but there are no reports of these receptors forming heterodimers with each other. This experimental finding is in agreement with the prediction made by the model and suggests that dimerization interactions can be used to predict some aspects of receptor cross-talk.

4.3. Implications for drug design

Drug design at the receptor level has historically focused on optimizing the drug's ability to bind to and activate a receptor. However, the results presented here suggest that a ligand's ability to induce homo- or heterodimerization could also provide a novel route to control other cellular properties such as receptor localization. This localization, in turn, could affect signaling processes such as receptor cross-talk, which was discussed above, or internalization. For example, one could envision designing a drug that induces heterodimerization and internalization. By dosing with this drug, one would not only internalize the primary target receptor, but also the dimerization partner of the receptor.

Therefore, a potentially powerful and as yet unexplored option for drug design would be to tailor ligands to control receptor dimerization. High throughput screens for such drugs could be performed using a FRET type assay in a whole cell system. The simulations done here suggest that drug-induced modulation of receptor dimerization should have the most pronounced effects under slow diffusion and high receptor density conditions. Similar results may be possible for other non-G-protein coupled receptors, assuming that receptor dimerization and activation are sufficiently decoupled.

In sum, by looking at protein dimerization we begin to address a larger problem of how the kinetics of protein–protein binding affects the overall organization of the membrane. Related research in the field of diffusion-limited aggregation has shown that diffusion and binding together can yield intricate structures [31] from simple mechanisms. We expect that protein–protein interactions such as dimerization are also capable of forming such structures, which in turn affect cellular physiology.

Although dimerization between monomeric proteins is only one kind of binding interaction, we suggest that it represents a basic building block of protein localization. We have demonstrated that by dimerization alone, membrane proteins can exhibit long-range clustering depending on the kinetics of dimerization relative to the diffusion rate. Other localization mechanisms, such as multivalent interactions and lipid rafts, clearly also play a role in controlling a proteins location; however, the role of dimerization in localization has until now been overlooked.

Acknowledgments

This work was supported by a Whitaker Foundation Fellowship, NIH Training Grant # GM08353 (P.J.W.), and NIH R01 GM62930-01 (J.J.L.).

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