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Unraveling the impact of lipid domains on the dimerization processes of single-molecule EGFRs of live cells

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ABSTRACT

Epidermal growth factor receptor (EGFR/ErbB1) is a transmembrane protein that can drive cell growth and survival via the ligand-induced dimerization of receptors. Because dimerization is a common mechanism for signal transduction, it is important to improve our understanding of how the dimerization process and membrane structure regulate signal transduction. In this study, we examined the effect of lipid nanodomains on the dimerization process of EGFR molecules. We discovered that after ligand binding, EGFR molecules may move into lipid nanodomains. The lipid nanodomains surrounding two liganded EGFRs can merge during their correlated motion. The transition rates between different diffusion states of liganded EGFR molecules are regulated by the lipid domains. Our method successfully captures both the sensitivity of single-molecule processes and statistic accuracy of data analysis, providing insight into the connection between the mobile clustering process of receptors and the hierarchical structure of plasma membrane.

1. Introduction

Live cells must execute a variety of cellular processes to survive in a changing environment. These cellular processes can be organized into three networks: the signaling network, transcription regulation network, and metabolic network [1–5]. The signaling network is responsible for relaying messages from the external environment to the cellular nucleus. The first event of signaling processes occurs at various types of receptors on the plasma membrane of a live cell. One such receptor is the epidermal growth factor receptor (EGFR/ErbB1), which drives cell growth and survival [6]. EGFR signaling is also responsible for several disease pathogeneses [7].

EGFR is a transmembrane protein containing an extracellular binding domain, a single transmembrane domain, and a cytoplasmic tyrosine kinase domain. Ligands such as epidermal growth factor (EGF) can bind to the extracellular domain and thus stimulate conformational changes in EGFR that promote receptor dimerization and the activation of the intracellular tyrosine kinase domain [6,8]. The phosphorylated tyrosine residues then act as docking sites for adaptor proteins, which further activate intracellular signaling cascades.

EGFR can form either a homodimer with an EGFR or heterodimers with other members of its receptor-family [8–10]. However, conclusions about the size of these molecular aggregates and the ligand-occupancy of the signaling complex remain controversial. Conventional steady-state ensemble approaches cannot be used to address the stochastic nature of receptors that encounter each other in the highly heterogeneous and fluidic plasma membrane. Recent advances in single-molecule fluorescent imaging and tracking have provided more insights into the behavior of intact EGFR on the plasma membranes of live cells [11–14]. Multiple EGFR signaling complexes have been found with varying degrees of stability. For example, by analyzing single-molecule trajectories the dissociative rate constants of dimers, $k_{off} = 1.24s^{-1}$, $k_{off} = 0.74s^{-1}$, and $k_{off} = 0.27s^{-1}$, have been determined for unoccupied, singly, and doubly liganded dimers, respectively [13]. It was found that dimers composed of two ligand-bound receptors are the most long-lived; their dissociative rate is more than four times slower than that of the unoccupied dimers. The association of EGF to monomer, unliganded dimers, and singly liganded dimers also differs. The doubly liganded dimers can enter into a very slow-moving state that correlates directly with receptor activation [13].

Recently, Lidke and his coworkers devised a two-color quantum-dot tracking method to visualize the state-dependent dimerization processes of human EGFR [13]. A three-state hidden Markov model was proposed to deduce the transition rates between free, co-confined, and dimerized states. They found that disruption of actin networks leads to the faster diffusion of receptor dimers and concluded that actin corrals establish the confinement zones for EGFRs. Sequestering cholesterol to disrupt lipid domains was found to have a minimal effect on the diffusion of EGFR dimers [13], suggesting that lipid domains have a negligible role in the confinement of EGFRs. However, as the native protein–lipid architectures and dynamics in the membrane environment are far

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from well understood, the existence and resulting functionality of a lipid nanoscale structure on EGFR dimerization remains controversial. In this study, we address the issue of the effect of lipid nanodomains on the dimerization process of EGFR molecules.

2. The energetic model

The plasma membranes of live cells are complex and highly heterogeneous [15–18]. Single-molecule tracking of a receptor protein can effectively probe into the microscopic environment and thermal fluctuations in a living cell. The influences of cellular objects or structures far separated from a receptor protein are negligible in the description of single-molecule diffusion of the protein. Thus, we can focus on the local environment of the protein. Based on our current knowledge of single-molecule diffusion in the plasma membrane, two types of interactions between a receptor protein and its local environment shall be taken into account. Firstly, the receptor protein can induce a local ordering of the surrounding lipid molecules via a lipid–protein interaction [19–21]. The receptor can also serve as a nucleation site to form a stable lipid domain and results in a free energy decrease. Secondly, there exist actin skeleton-induced membrane compartments [22,23]. Our model incorporates a cooperative action with the hierarchical structure of actin skeleton-induced membrane compartments, protein-induced lipid ordering domains and dynamic diffusion of receptor proteins. The basic ideas of the model are illustrated in Fig. 1(a) including the protein-induced lipid ordering and the actin skeleton-induced membrane compartment with length scales λ_x and λ_y.

The diffusion of a receptor protein in the plasma membrane can be described with the generalized Langevin equation [24]:

$$m\gamma\partial_t\vec{x_k} = -\nabla_k(\mathbb{V}+\mathbb{F}) + \overrightarrow{f_k} = -m\gamma\mathbb{U} + \overrightarrow{f_k}. \quad (1)$$

Here $\vec{x_k}$ is the position vector of the k-th receptor molecule and γ is the frictional parameter, which relates to the diffusion coefficient by $m\gamma = k_BT/D$. The fluctuation force $\overrightarrow{f_k}$ on the molecule fulfills the fluctuation-dissipation theorem $\left\langle \overrightarrow{f_k}(t)\overrightarrow{f_k}(t+\tau)\right\rangle = 2k_BTm\gamma\delta(\tau)$. We used a potential profile of $\mathbb{V} = \mathbb{V}_0 cos^n\left[\pi\left(\frac{x_k}{\lambda_x}+\frac{y_k}{\lambda_y}\right)\right]cos^n\left[\pi\left(\frac{x_k}{\lambda_x}-\frac{y_k}{\lambda_y}\right)\right]$ to model the interaction between the protein and actin corral with different gradients. By adjusting the exponent n and $\mathbb{V}_0$, we can control the interaction strength and range of the actin confinement in a membrane compartment.

We consider the lipid-dependent segregation of a mixture of raft and non-raft lipids as a lipid domain with an order parameter $\phi(r)$, which indicates the degree of enrichment of raft lipids. The enriched lipid rafts are $\phi(r) > 0$ (inside the red dashed circle in Fig. 1(a)) and the depleted raft lipids are $\phi(r) < 0$ (outside the red dashed circle in Fig. 1(a)). The dynamic evolution of the order parameter $\phi(r)$ follows the Cahn–Hilliard equation [25,26]

$$\partial_t\phi(r,t) = D\nabla^2\left[\partial_\phi\mathbb{F}\right] \quad (2)$$

with $\mathbb{F}$ denoting the Ginzburg–Landau functional of the lipid–lipid and protein–lipid interaction energy densities [27]. $\mathbb{F}$ can be expressed as

$$\mathbb{F} = \int\left[\frac{1}{2}\alpha\phi(r,t)^2 + \frac{1}{4}\beta\phi(r,t)^4 + \frac{1}{2}\chi|\nabla\phi(r,t)|^2 - \phi(r,t)S_P(r)\right]dA. \quad (3)$$

The Cahn–Hilliard equation is a coarse-grained version of Brownian-like diffusion [26]. According to the Landau mean field theory [25] of a physical system with an inversion symmetry $\phi(r) = \phi(-r)$, the first two terms of Eq. (3) describe the thermal stability of the system. The parameter α represents the binding energy of lipids (i.e., the energy needed to remove a lipid molecule from the lipid membrane), β denotes the interaction strength between lipid molecules, and the third term is

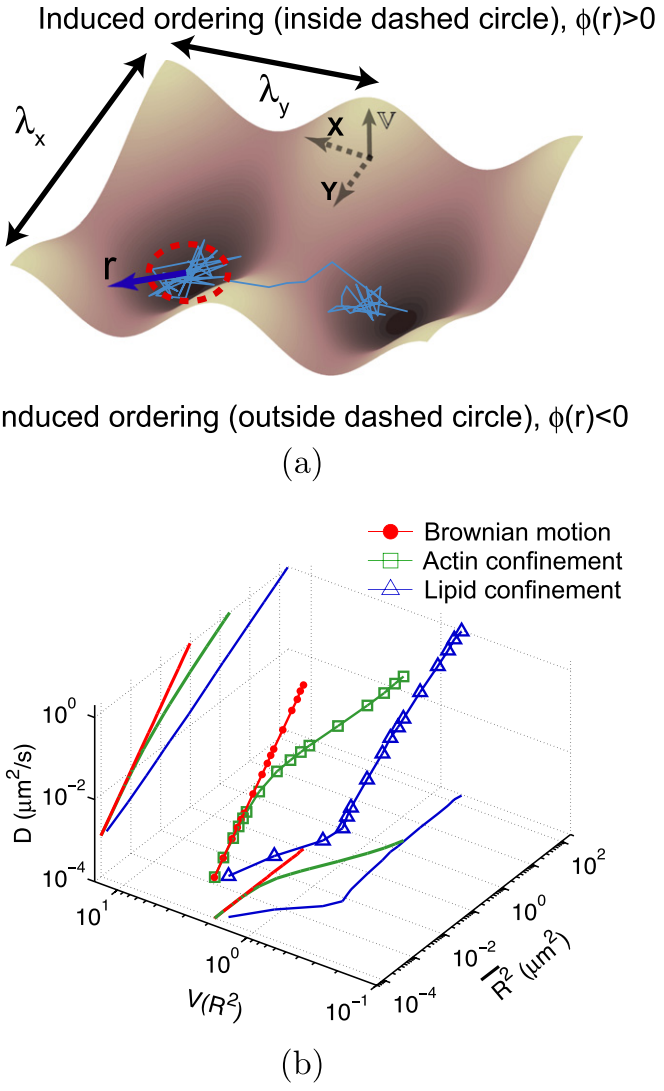

Fig. 1. Model and simulation result. (a) Schematic showing the model that involves the structure of actin skeleton-induced membrane compartments, protein-induced lipid ordering domains and dynamic diffusion of protein complexes. The actin skeleton is modeled with a potential barrier ($\mathbb{V}_0$) and the length scales (λ_x and λ_y). The region with induced lipid ordering (inside the red dashed circle) has $\phi(r) > 0$ for enriched raft lipids and outside has $\phi(r) < 0$ for depleted raft lipids. (b) Simulation results of $V(R^2)$ are plotted in the $V(R^2)) - R^2 - D$ space for single-molecule receptors under free Brownian motion (red solid circles), under confined diffusion by either actin corrals alone (green open squares, $\lambda_x = \lambda_y = 70nm$, barrier height $\mathbb{V}_0 = 0.5$ eV, and $n = 1$) or both actin corrals and lipid domains (blue open triangle). The initial condition of the lipid environment was assumed to be a homogeneous mixture with 40% raft lipids and 60% non-raft lipids ($\phi_0 = -0.2$). The length scales, confinement amplitude, and exponent of the actin corrals are $\lambda_x = \lambda_y = 70nm$, barrier height $\mathbb{V}_0 = 0.5$ eV, and $n = 3$.

from a line tension at the boundary of two different lipid phases. Typical values of the parameters are $\alpha = k_BT/2$, $\beta = k_BT/3$, and $\chi = 4k_BT\cdot\mu m^2$ for the plasma membrane system of live cells. The presence of a protein results in a force field that breaks the symmetry of lipid phases. The symmetry breaking yields a linear term with negative value in $\mathbb{F}$ [25]. Therefore, the lipid ordering $\phi(r) > 0$ induced by a protein ($S_P(r) = 1$) decreases the free energy of a lipid domain.

To evaluate the effect of the heterogeneities of plasma membrane on the movement of a receptor molecule, we solved Eqs. (1) and (2) self consistently to yield single-molecule trajectories and then calculated the variance $\sigma^2_{R^2}$ of the squared displacements $R^2(t)$. We present our simulation results in a plot of $V(R^2)$ versus $\overline{R^2(t)}$ with $V(R^2) = \sigma^2_{R^2}/$