PNAS COMMENTARY

Membrane defects as a generalized driving force for membrane protein interactions

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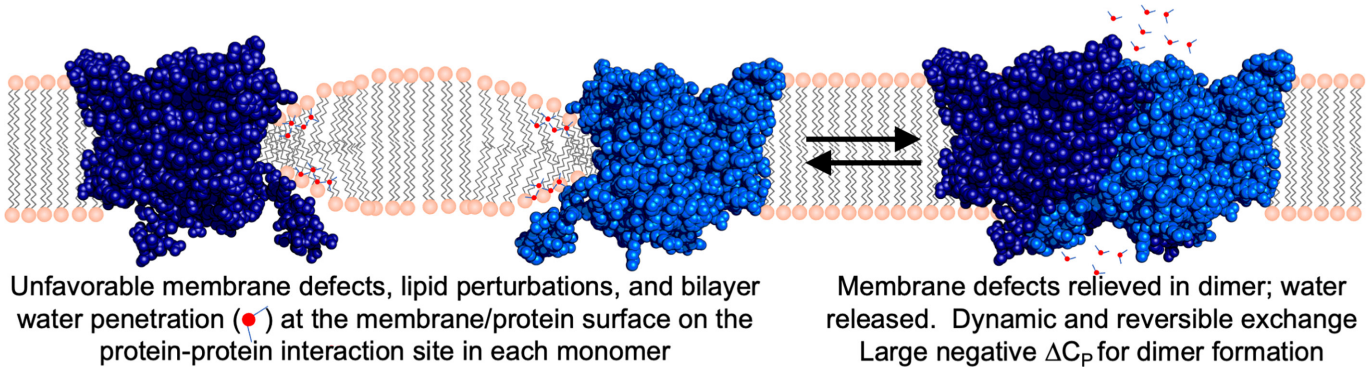

Fig. 1. Schematic of CLC-ec1 dimerization reaction and potential molecular driving forces. Two CLC-ec1 monomers (light blue, dark blue) dynamically interact at their "short" protein interface to form a dimer with high shape complementarity. This dimerization interface is exposed to lipid in the monomeric state where it causes the formation of a membrane defect. This defect is a local, high-energy conformation where the bilayer thins to match the hydrophobic thickness of the monomeric protein at this site. Lipids are deformed in this perturbed membrane, and increased water enters the bilayer (12). This membrane defect is absent in the dimer structure because the two short, protein–protein interfaces are sequestered away from lipid. Dimerization is associated with a large negative heat capacity change.

How can the hydrophobic effect drive binding between nonpolar protein interaction surfaces buried within the nonpolar regions of the membrane? Compared to bulk, the water concentration is at least a million-fold lower in the central bilayer region (1), which means that there is essentially no water to be released. Paradoxically, a thermodynamic analysis of the CLC-ec1dimerization reaction published in PNAS (2) reveals a high negative heat capacity change upon dimer formation, a hallmark signature of water release. Is the hydrophobic effect a generalized force driving bilayer interactions of membrane proteins?

In membrane protein folding, we expect the hydrophobic effect to manifest its power upon insertion of polypeptides into the bilayer, and this reaction is the basis for the prediction of transmembrane segments from primary sequence using hydrophobicity scales (3). But once inserted, it has long been posited that interactions in membrane proteins would be primarily mediated by other forces within and between protein interfaces such as van der Waals packing, hydrogen bonding, and electrostatic interactions (4). Increases in lipid chain entropy will also occur as the surface area buried away from lipids decreases when membrane proteins associate. But none of these have ever been expected to involve the release of water. Still, due to the technical challenges associated with experimentally measuring membrane protein association reactions, very little data have been available to examine these tenants.

Chadda et al. have been systematically overcoming this barrier using the dimerization equilibrium of the *Escherichia coli* chloride/proton antiporter (CLC-ec1) (5–7). Notably, the CLC-ec1 membrane protein is not a toy system. Rather, it is a large, native *E. coli* protein containing multiple transmembrane domains in each protomer. ClC-ec1 functions as both a monomer and a dimer, and it is structurally well characterized as the X-ray crystal structures are known for both forms (5, 8). The dimerization interface is formed through association with a large so-called "greasy" interface composed entirely of nonpolar side chains. The protein–protein interface displays high shape complementarity (5), suggestive of highly favorable van der Waals packing. Consistent with this idea, the Robertson group showed in previous work that the dimerization free energy change was on the order of −11 kcal per mole in 2:1 POPE:POPG liposomes using a standard state of 1 subunit per lipid (9). This corresponds to a population of essentially 100% dimer at physiological expression levels.

What are the forces driving such stable dimer formation? In PNAS (2), the authors execute a van't Hoff analysis to further investigate the origins of CLC-ec1 dimer stability. This classic experiment examines the temperature dependence of the equilibrium constant to reveal the underlying enthalpic and entropic contributions. As a prerequisite to this tour-de-force analysis, the authors establish that CLC-ec1 exists in a dynamic

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Author contributions: K.G.F. wrote the paper.

The author declares no competing interest.

See companion article, "A thermodynamic analysis of CLC transporter dimerization in lipid bilayers," 10.1073/pnas.2305100120.

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Published October 18, 2023.

assembly state in membranes where it can exchange subunits. Even though the equilibrium position exists far in favor of the dimer, the subunits are not kinetically trapped. They are actively swapping subunits, and the average population is dictated by thermodynamic considerations. This fundamental thermodynamic finding means that alterations to the environment, temperature, pressure, and most importantly the membrane solvent, can—and do—influence the oligomeric state. Indeed, the CLC-ec1 population shifts due to perturbations of the membrane composition are not subtle. The dimer stability is 2.3 kcal mol^{-1} less stable in 2:1 POPE:POPG as compared to *E. coli* polar lipids, which corresponds to a ~50-fold shift in the population towards monomer. Herein lies a significant result as the biological implications of lipids modulating membrane protein–protein interactions could be profound.

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The response to temperature is not subtle either. In this work, Chadda et al. take advantage of the wide temperature range over which many membrane proteins are stable (10) and measure both the function and monomer–dimer equilibrium constants over a range of 31 to 62 °C. Controls show that neither membrane bilayer environment undergoes any phase transitions over this same temperature range. Thus, the temperature dependence of the CLC-ec1 equilibrium constant is a manifestation of the protein association itself.

Figure 4G in PNAS (2) shows the key result clearly demonstrating a nonlinear dependence of the equilibrium constant on temperature. This downward curvature of a van't Hoff plot reveals not just the enthalpic and entropic contributions but also demonstrates a large negative heat capacity upon dimerization. Both the magnitude and sign of the ΔC_P are reminiscent of the hydrophobic effect as observed in soluble protein folding. The crystal structures of the monomer and dimer indicate that there are no large conformational changes associated with the monomer-to-dimer transition. Therefore, there is no obvious protein structural transition to be invoked to explain the ΔC_P. Moreover, given that the protein interface remains embedded in the bilayer as evidenced by the functional assays, how is such a large ΔC_P even possible?

Molecular interpretations of ΔC_P are still quite challenging (11); however, the authors offer a number of potential suggestions. A principal one derives from the observation that the interaction interface on the monomeric protomer has a short hydrophobic thickness as compared to the membrane. Molecular dynamics simulations show local membrane defects such as overall thinning, perturbations to lipid acyl chain packing, and increased water near this protein surface, but only in the monomeric form (12, 13) (Fig. 1). These high-energy defects disappear when CLC-ec1 dimerizes. Thus, relaxation of membrane defects is a driving force for membrane protein interactions within the bilayer. To the extent that these defects perturb water structure at or in the membrane, the hydrophobic effect can, in fact, possibly be said to be promoting "greasy interactions in a greasy environment". Further, it is energetically favorable for two monomers to combine their respective defects into one single defect even before the protein–protein contacts are formed (13). Thus, one can easily imagine that a membrane defect driving force is a general function operating in a large number of cellular contexts. Conversely, short lipids that match the short interface on the monomer can mitigate the membrane defect and stabilize the monomer. Perhaps there are even unknown small lipids that cells use to accomplish this very task.

Hydrophobic mismatch is not new (14–17), but in PNAS (2), we see the mighty force that the local defects can wield. Could this be true for membrane proteins in general whenever there is a mismatch between the nominal hydrophobic thickness of the bilayer and the protein structure? Have many membrane protein structures evolved to take advantage of this membrane material property? The thermodynamics of the CLC-ec1 dimerization reaction put these questions and more on a firmer foundation.

ACKNOWLEDGMENTS. K.G.F. work is supported by NIH grant R35GM148199 and NSF grant MCB1931211.

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