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Membrane protein takes the brakes off:

An enzyme family has evolved to distort membranes and diffuse quickly to find substrates

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Proteases are enzymes that use water to break amide bonds of protein substrates. This process—proteolysis—plays a myriad of roles from digestion to cell signaling, and regulation of protease functions are critical to all life forms. A fascinating class of proteases have their water-wielding active sites immersed in the water-repelling (hydrophobic) environment of the lipid bilayer and cleave the transmembrane regions of their substrates. How these intramembrane-cleaving proteases (I-CLiPs) (1) carry out this apparently paradoxical process has intrigued biochemists for 20 years. Among the challenges is deciphering how I-CLiPs diffuse through viscous cell membranes crowded with other membrane proteins to find their substrates. On page 497 of this issue, Kreutzberger *et al.* (2) find that one class of I-CLiPs, the rhomboid family, diffuses much faster than other membrane proteins, in violation of estimated speed limits for their size. This implies that these membrane proteins have evolved for rapid diffusion to carry out their functions effectively.

The rhomboid I-CLiP family is defined by the presence of a serine amino acid in its membrane-embedded active site, and this serine is directly involved in proteolysis of rhomboid substrates. Among I-CLiPs, rhomboids are particularly well studied because they have yielded more readily to crystallographic elucidation of structure with atom-level resolution (3). Moreover, rhomboid structures have been captured in several different conformational states (4). Rhomboid I-CLiPs are found in virtually all life forms, and cleavage of their substrates plays a variety of roles, including cell signaling, mitochondrial function, and virulence of certain human pathogens (5). Yet, despite their often-critical functions, rhomboids and other I-CLiPs process their substrates excruciatingly slowly in comparison to other enzymes, raising questions about how proteolysis in the membrane is regulated.

The molecular reasons for the sluggish substrate recognition and cleavage have remained mysterious. One idea is that the binding and unwinding of the transmembrane domain of the substrate from a classical α-helical conformation, necessary for water to have access to the amide bond for breakage, simply takes time. In the absence of any apparent need for an energy molecule such as adenosine triphosphate (ATP) to drive the confonnational changes in enzyme and substrate, rhomboid I-CLiPs are thought to rely on their own random motions to eventually unwind and cleave their helical substrates.

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Previous work demonstrated that rhomboid I-CLiPs have weak substrate affinity and that instability of the substrate transmembrane α helix determines ready access to the active site for cleavage (6). But this “stop- and-frisk” model requires considerable time, because rhomboid I-CLiPs would have to diffuse through the lipid bilayer and interrogate many other membrane proteins in search of potential substrates. Thus, diffusion of rhomboid I-CLiPs through the cell membrane might be the rate-limiting factor in substrate proteolysis. This idea is counter-intuitive: Water-soluble enzymes that are diffusion-limited carry out catalysis more efficiently by many orders of magnitude and are considered “catalytically perfect.” That is, catalysis is so efficient that aqueous diffusion, although extremely rapid, determines the rate of substrate conversion to products. Could rhomboid I-CLiPs be catalytically perfect in cell membranes?

To measure diffusion through membranes, Kreutzberger *et al.* engineered the fusion of rhomboid I-CLiPs with a self-labeling protein capable of incorporating a small fluorescent molecule. The fluorescence-tagged protein was visualized in living cells using a microscopic technique that provides single-molecule resolution to make movies tracking fluorescent rhomboid I-CLiP movements within lipid bilayers. As a result, they determined that rhomboid I-CLiPs diffuse faster through membranes than other multipass membrane proteins, even faster than rhodopsin, the previous record holder (7). More surprising was the finding that the rate of rhomboid I-CLiP diffusion defies a long-established correlation between protein radius and diffusion of membrane proteins (8).

How can rhomboid I-CLiPs break this apparent speed limit for diffusion of membrane proteins? The key is apparently the shape and thickness of the band of hydrophobic amino acids on the surface of the folded rhomboid I-CLiP protein that are embedded in the membrane. Following the chemical principle of “like dissolves like,” hydrophobic amino acids on the surface of a membrane protein are typically found in the lipid bilayer, whereas water-loving (hydrophilic) amino acids are typically exposed to the aqueous environment outside the membrane. For most membrane proteins, the thickness of this hydrophobic band matches that of the membrane. However, rhomboid I-CLiPs have a thinner hydrophobic band, a mismatch with the membrane thickness that can lead to membrane distortion (9). Presumably, the membrane distortion, in turn, reduces the membrane viscosity experienced by the rhomboid I-CLiP, allowing it to move more freely through the membrane. Thus, rhomboid I-CLiPs act like “icebreakers” to speed their own passage through the viscous lipid bilayer (see the figure).

Membrane distortion also occurs through mismatch in shape: Certain surface rhomboid I-CLiP amino acids apparently do not fit well in the lipid bilayer, disrupting the otherwise orderly arrangement of membrane lipids. A key region of rhomboid I-CLiP enzymes is responsible for immersing these disrupting amino acids in the membrane, and deletion of this region of the protein leads to slower membrane diffusion, even though a smaller protein typically results in faster membrane diffusion.

The rate of rhomboid I-CLiP diffusion through the membrane has functional consequences. Speeding up or slowing down rhomboid I-CLiPs in cell membranes affects the rate of substrate proteolysis, suggesting that diffusion through the lipid bilayer is the rate-

determining step in rhomboid I-CLiP substrate cleavage. These proteases have apparently evolved for faster diffusion because this is critical for sufficient proteolysis and signaling for proper development and maintenance of the organism. However, even catalytically inactive rhomboid proteins—membrane protein chaperones called iRhoms (10, 11)—diffuse rapidly through lipid membranes, suggesting that facile diffusion is critical to their function as well.

These new findings should stimulate a rethink in membrane biophysics about properties critical for membrane-protein function. What other membrane proteins might have evolved to break the viscosity-imposed speed limit of diffusion through lipid bilayers? Is this property rate-limiting with respect to function? Can algorithms be developed to facilitate the identification of these lead-footed membrane proteins? Perhaps a good starting point would be investigating other I-CLiP families. Time will tell whether the rhomboid superfamily is an anomaly or the first of many other membrane proteins that distort the membrane to race through the lipid bilayer in search of protein partners. ■

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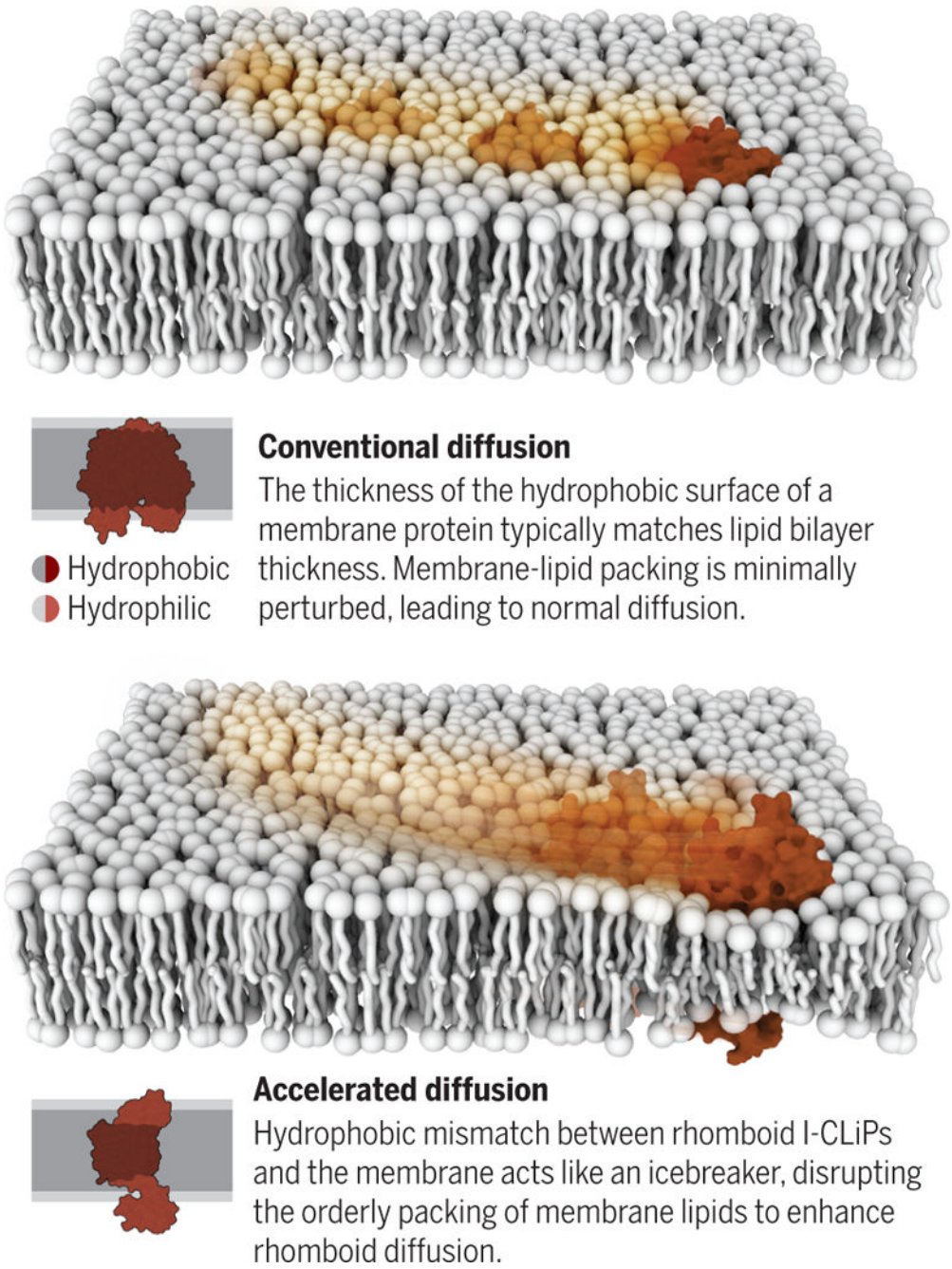

Figure. Rhomboid I-CLiPs distort the lipid bilayer

Proteins embedded in the lipid bilayer move in two dimensions within the membrane. Features of the protein that interfere with lipid packing increase diffusion in the membrane.