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The Life of a Membrane Protein

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Our understanding of the true nature of cellular membranes has changed much since the "Fluid Mosaic" model by Singer and Nicolson (1), which treats the membrane as a two-dimensional fluid in which lipids and proteins diffuse freely. We now know that, in fact, membranes are "more mosaic than fluid" (2), in the sense that lipids and proteins are not diffusing freely but instead associate within and between species on many scales, creating domains of distinct composition different from the neighboring domains. The membrane and its constituents cannot be modeled as an equilibrium system; it is "dynamic, yet structured" (3). We also know that membrane proteins interact with each other, with lipids, and with the cytoskeleton underlying the membrane in various and complex ways (4), leading to a myriad of diffusion modalities in an extremely heterogeneous environment. The heterogeneity of biological membranes is not only spatial but also temporal, with interactions between membrane constituents taking place on timescales ranging from milliseconds to seconds and more. To complicate things even more, we learned that membrane lipids and proteins are continuously being recycled, delivered to specific areas, and withdrawn from other areas of the membrane by endo- and exocytosis. As a result, the concentration gradients driving diffusion are changing in time and can lead to membrane patchiness (5,6). The rates of recycling also vary widely from tens of microseconds to minutes depending on cell type.

The authors of this manuscript make the point that the apparent diffusional behavior in such environments is highly dependent on the temporal resolution of the measurement and on the duration of the experiments observing diffusion. This is so because diffusion may look different at different time scales and over different periods. Specifically, some of the most commonly used measurement and analysis methods (such as time-averaged mean-squared displacement) are obscuring details of processes that evolve over time. Thus, assumptions such as "diffusion measured over one temporal or spatial range will be identical to diffusion measured over different temporal or spatial ranges" (termed scale-invariant) or "every region of space is visited with equal probability" (termed ergodic) are not only not substantiated but in fact untrue in some cases.

The authors proceed to suggest using a continuous-time random walk approach to analyze single-particle tracking trajectories, a model that does not assume scale invariance and ergodicity and thus is more appropriate for analysis of non-Brownian diffusion.

To demonstrate the insights that can be gained using this approach, they present the careful study of CD93, a one-transmembrane-domain membrane protein. It seems that several populations (diffusion-wise) exist on the plasma membrane, showing different modes of diffusion at different timescales. The notion of "aging" used by the authors is probably the most intuitive way to convey the idea of a process, an evolution, in the diffusion domain. The way a protein diffuses over short time durations is not how it diffuses over long durations. To stress this point: subdiffusion, for example, is a mode of diffusion with a "diffusion coefficient" that decreases with the duration of the measurement. Or, in other words, the dependence of the mean-square displacement (MSD) on time is less than linear. The "diffusion coefficient," D, defined as the slope of the MSD versus lag-time plot, actually loses its common meaning, because D itself depends on time in non-Brownian cases such as this. So, if one mentions the "diffusion coefficient" of a subdiffusing protein, one should also mention "when," or else it has no meaning. This, however, is not what "evolution" in the context of this study means. This is so because typically subdiffusion can be described with a power law (MSD $\propto t^{\alpha}$) in which the power (α) does not evolve over time. So, even though the "diffusion coefficient" evolves over time, the mode of diffusion does not.

In contrast, the analysis presented here demonstrates that CD93 modes of diffusion evolve over time, that the

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power of t is itself a function of time (noted as α_τ). Thus, CD93 proteins that initially diffused freely become increasingly corralled so that at any given time, two populations exist with high mobility and low mobility (corralled), with ~20% of the CD93 that was originally freely diffusing becoming corralled after 8 s of observation. The corralled CD93 remains corralled (there is no escape from corrals), and thus no replenishment of the freely diffusing population is expected, which would inevitably lead to the confinement of all CD93 in corrals. The fact that the freely diffusing population does not disappear must be explained by endocytosis and exocytosis of CD93. And, indeed, the authors proceed to prove exactly this process. The two mechanisms together, i.e., stable (and terminal) trapping of originally freely diffusing CD93 and endocytosis of trapped protein followed by exocytosis of newly synthesized (and freely diffusing) protein maintains a balance between a high-mobility, "young" population and a trapped "aging" population.

Born free, CD93 is gradually getting entrapped in corrals limiting its mobility; unable to escape, it awaits its inevitable endocytosis.

A strong message of this work is the importance and the power of careful, appropriate, and precise modeling of diffusion properties. The detection of the fact that CD93 should all be frozen (trapped) according to the diffusion analysis alone is what triggers the search for additional mechanisms that can explain why this is, in fact, not the case. Alongside examples of other proteins, such as the type-I MHC-I (7) and the multispan Kv2.1 potassium channel (8), this study shows another important example in which dynamic recycling of the membrane constituents, together with intricate diffusion modalities, sculpt the "face" of the cell. Surely, this concept is likely to produce additional new examples and will become a regular part of the way we understand cell membranes.

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