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In Vitro Membrane Platform for the Visualization of Water Impermeability across the Liquid-Ordered Phase under Hypertonic Conditions

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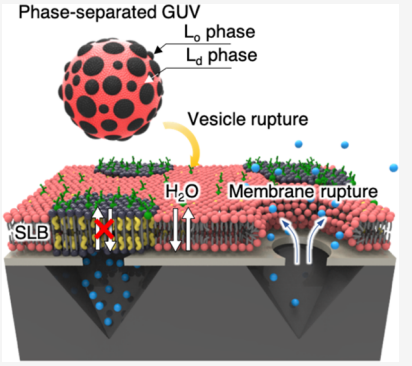
ABSTRACT: Passive water penetration across the cell membrane by osmotic diffusion is essential for the homeostasis of cell volume, in addition to the protein-assisted active transportation of water. Since membrane components can regulate water permeability, controlling compositional variation during the volume regulatory process is a prerequisite for investigating the underlying mechanisms of water permeation and related membrane dynamics. However, the lack of a viable in vitro membrane platform in hypertonic solutions impedes advanced knowledge of cell volume regulation processes, especially cholesterol-enriched lipid domains called lipid rafts. By reconstituting the liquid-ordered (L_o) domain as a likeness of lipid rafts, we verified suppressed water permeation across the L_o domains, which had yet to be confirmed with experimental demonstrations despite a simulation approach. With the help of direct transfer of the L_o domains from vesicles to supported lipid membranes, the biological roles of lipid composition in suppressed water translocation were experimentally confirmed. Additionally, the improvement in membrane stability under hypertonic conditions was demonstrated based on molecular dynamics simulations.



1. INTRODUCTION

The penetration of water molecules across cell membranes, a vital step in maintaining cellular viability under continually altered environmental conditions, plays an essential role in life-sustaining cellular activities such as cell volume control, proliferation, angiogenesis, and cell migration. An endless repetition of water influx/outflux over the lipid membrane boundary serves as a compensative regulatory mechanism for the homeostatic balance of the levels of glucose, acids, calcium, and fluid volume.[1] However, unstable water penetration across cell membranes unbalances structural/functional homeostasis, resulting in cellular stress, which may be associated with diverse intracellular mechanisms for unfolded protein responses, cell senescence, and DNA damage responses.[2] Besides, consequential volumetric changes result in cell swelling and shrinkage, leading to membrane disruption and eventual cell lysis.[3,4] In general, such cellular activity arises from water translocation across the lipid membrane boundary via a regulatory volume control mechanism. Thus, proteins that selectively transport ions (e.g., Na+, K+, and osmolytes) or water through aquaporins have been extensively explored.[5] Additionally, the translocation of water can be realized via diffusion under a concentration gradient of osmolytes[6] until the concentration gradient reaches zero over the lipid membrane. Thus, it is important to explore spontaneous water permeation driven by the internal composition of the lipid membrane, unaided by protein channels.

Water permeability varies significantly from cell to cell depending on lipid composition, the population of protein channels,[5] or temperature.[7] For example, high permeability denotes fluid lipid bilayers or expression of more aquaporins or high temperatures, whereas low water permeability implies the opposite. Over centuries of scientific discovery, the analysis of dying cells by imbalanced water permeation has not been rigorously explored owing to (i) intrinsic complexity and protein existence aiding volume controls that suppress the identification of water permeation via lipid compositions or (ii) a lack of systematic in vitro membrane platforms for exploiting the membrane reconstructed under ionically asymmetric environments (i.e., hyper/hypotonic solutions). Instead, molecular dynamics (MD) simulation-based evaluation of molecular permeability has been intensively explored, as it unveils a variety of membrane-associated biological activities, including molecular entrance, translocation, exit of simple permeants, etc.[8,9] In particular, two-dimensional (2D) compositional inhomogeneity caused by localized cholesterol-enriched liquid-ordered (L_o) domains, a likeness of lipid rafts, is one of the primary subjects of simulation studies owing to

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