

# Analysis and Classification of Directional Motions of Cholesterol Crystals Surfing on a Lipid Membrane

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Cholesterol crystallization within membrane environments involves a complicated interplay of cholesterol molecules with adjacent phospholipids, cholesterol molecules, and surrounding water layers. Using a mixture of phospholipid and cholesterol at supersaturating concentrations, certain directional movements, or “swimming” behavior, of cholesterol crystals within the cholesterol-laden membrane media were demonstrated, which were distinctive from non-directional diffusion. Using real-time epifluorescence microscopy, the gradual solvent exchange from a 50% organic medium to an aqueous buffer producing contiguous supported lipid bilayers with multiple types of three-dimensional cholesterol crystals undergoing different pathways of structural evolution was observed. These movements were defined in five translational motions: “parking”, “curve”, “spiral”, “back-and-forth”, and “circle” trajectories, all reminiscent of nanocars. These directional movements featured short-lived intermediate crystal structures surfing on the membrane while concomitant crystal growth occurred. In all cases, the directional swimming movements were transient and terminated after a certain time by associating with the membrane or surface, which was confirmed by observing both cholesterol and phospholipid-labeled media. It is speculated that the attraction between the cholesterol crystals and the surrounding cholesterol molecules drives crystal growth and movement and demonstrates how modulating molecular interactions between cholesterol crystals and lipid membranes can impart car-like direction motions in potentially deterministic manners.