report

Rafts defined: a report on the Keystone symposium on lipid rafts and cell function

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Abstract The recent Keystone Symposium on Lipid Rafts and Cell Function (March 23–28, 2006 in Steamboat Springs, CO) brought together biophysicists, biochemists, and cell biologists to discuss the structure and function of lipid rafts. What emerged from the meeting was a consensus definition of a membrane raft: "Membrane rafts are small (10–200 nm), heterogeneous, highly dynamic, sterol- and sphingolipid-enriched domains that compartmentalize cellular processes. Small rafts can sometimes be stabilized to form larger platforms through protein-protein and protein-lipid interactions." This definition helps to clarify current thinking in a field that has been plagued by the heterogeneous and sometimes ephemeral nature of its subject.— Pike, L. J. **Rafts defined: a report on the Keystone symposium on lipid rafts and cell function. *J. Lipid Res.* 2006.** 47: **1597–1598.**

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It is rare that a meeting is held to discuss an entity that is difficult to visualize, has an ill-defined molecular composition, and whose very existence has been questioned (1). Such was the case in Steamboat Springs, CO, on March 23–28, 2006, when the Keystone Symposium on Lipid Rafts and Cell Function was convened. Organized by Linda Pike (Washington University) and Michael Edidin (Johns Hopkins University), the meeting brought together biophysicists, biochemists, and cell biologists to ponder the question, "What is a raft?"

Presentations ranged from the very biophysical talk given by Sarah Veatch (University of British Columbia) on phase behavior in model membranes to the biology-driven keynote address on the role of membrane domains in animal virus entry given by Ari Helenius (Institute of Biochemistry, Eidgenössisch Technische Hochschule, Zurich, Switzerland). Together, the discussions permitted the generation of a definition for "lipid rafts" in an ad hoc session on the final day of the meeting. All participants were invited to contribute to this effort, and the work product reflects the consensus of this broad-based group.

The definition adopted by the group was as follows: "Membrane rafts are small (10–200 nm), heterogeneous, highly dynamic, sterol- and sphingolipid-enriched domains that compartmentalize cellular processes. Small rafts can sometimes be stabilized to form larger platforms through protein-protein and protein-lipid interactions."

This definition was arrived at by listing all possible terms that could be used to describe lipid rafts, discussing and prioritizing them, and then working them into a definition for these domains. The terms that did not make it into the definition are at least as revealing of the state of the field as are the terms that did make the final cut. The definition is intended to apply specifically to microdomains in cells, not in model membranes, which are thought to be governed by a different, but overlapping, set of rules.

First and foremost, the term "lipid raft" was discarded in favor of the term "membrane raft." Although phase separation of lipids is acknowledged to provide an underlying energetic drive for the formation of membrane domains, the concept that the formation of membrane rafts is determined solely by lipid-driven interactions has been supplanted by the understanding that proteins and lipids both contribute to the genesis of these membrane microdomains.

Although "plasma membrane" was suggested for inclusion in the definition of membrane rafts, it was quickly excluded from consideration. Presentations and posters indicating the existence of raft-like domains on intracellular membranes such as the endoplasmic reticulum and mitochondria made it clear that the plasma membrane does not hold a monopoly on membrane domains. Although the nature of such "non-plasma membrane" membrane domains is not yet known, room must be left in the tent to welcome these newcomers.

This brings up the question of the typical lipid composition of a membrane raft. Although enrichment in cholesterol and sphingolipids was readily adopted as a characteristic of membrane rafts, this composition could

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be difficult to achieve in intracellular membranes that contain little if any of these two lipids. However, in her talk, Sarah Veatch noted that rather than disrupting lipid domains, cholesterol extraction from cells can increase the size of domains. Thus, it may be possible to generate membrane rafts in the absence of high concentrations of cholesterol.

In the context of the lipid composition of membrane rafts, the term "liquid-ordered" was considered and dismissed, largely on the grounds that there is, as yet, no solid evidence that cellular membrane rafts exist in this state. In a related judgment, the adjective "detergent-resistant" was considered but was soundly defeated. This outcome reflects the assessment of those in the field that detergent resistance is an artificial and highly subjective approach that can induce the formation of membrane domains and hence does not provide physiologically relevant information (2). Some individual proteins or complexes that can be shown by other methods to exist in rafts can be isolated through detergent-extraction procedures, but this approach for the de novo identification of raft components is no longer viable.

It came as no surprise that the term "small" was quickly brought up, and accepted, as a descriptor of rafts. Rafts have been getting smaller as the methods applied to study them have become more varied and more sophisticated. A host of imaging and analytical methods used to study membrane rafts were reported at the meeting. Included among them were electron microscopy alone or coupled with spatial statistics, heterofluorescence and homofluorescence resonance energy transfer, fluorescence quenching, fluorescence lifetime imaging microscopy, fluorescence recovery after photobleaching, fluorescence correlation spectroscopy, raster scan image correlation spectroscopy, and single particle tracking. The application of physical techniques that are capable of analyzing complexes in the range of tens of nanometers or less is an indication that the field has moved to a "less-is-more" view of membrane rafts.

But not too much less. Discussion swirled around the issue of how small (or how large) a raft can be. Complexes in the range of only a few nanometers, referred to as "lipid shells" (3) or "nanoclusters," were thought to be too small and potentially similar to thermodynamic fluctuations occurring near critical points in lipid phase diagrams (4, 5). At the other end of the scale, large complexes such as the immunological synapse (5), thought to be derived from the coalescence of multiple smaller domains, were deemed too large and too complex to be considered as "simple" membrane rafts. Thus, a range of 10–200 nm was adopted as the size of domains that would be considered to be rafts. The 200 nm upper limit was set to include the surface area (rather than simply the diameter) of the caveola, which was unanimously accepted as a member of the membrane raft family. This size range generates a cautionary note to those who would use immunofluorescence microscopy as a tool to study membrane rafts. As the size of rafts is smaller than the resolution of light microscopy, this methodology by itself cannot be used to colocalize proteins or lipids to the same membrane raft.

The notion of raft heterogeneity has long been accepted in the field (6) for a host of reasons, including the fact that proteins and lipids that are isolated together in the "raft" fraction of cells are actually localized in different areas of the cell (7). It is now understood that this heterogeneity is not limited to three-dimensional space but extends into the fourth dimension, time. Rafts may be transient or relatively stable, but all are viewed as dynamic structures. Several speakers (John Hancock, University of Queensland; Anne Kenworthy, Vanderbilt University; Ken Jacobson, University of North Carolina; Satyajit Mayor, National Centre for Biological Sciences, Bangalore, India) presented evidence that the concept of stable, preexisting lipid rafts is no longer tenable. Clustering of some proteins may occur because of differential affinity for or mobility in membrane microdomains. Alternatively, rafts may arise from the stabilization of otherwise transient, nanoscale domains, often in an actin-dependent manner. The concept of the stabilization of rafts to form larger platforms actually arose from discussions of raft size and the acknowledgment that some large domains, such as the immunological synapse or the entire brush border membrane, represent special cases in which small rafts are brought together, through protein-protein interactions, to produce a stable, biologically important structure.

A general consensus was that function was an important aspect of "raftness." Many different functional roles for rafts were described by speakers and in posters at the meeting (viral and toxin entry, cellular signaling, and protein and lipid trafficking). Although such specific functions were considered for inclusion in the definition of rafts, they were ultimately deemed to be too limiting. The idea that rafts are involved in the compartmentalization of cellular processes was felt to be more generally applicable and underscored the sense that, ultimately, it is functional significance that underlies the importance of being a raft.

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