Phospholipid signaling of geranylgeranyl-Rab5 peripheral membrane protein

E. Edler, E. Schulze, M. Stein

Max Planck Institute for Dynamics of Complex Technical Systems, Molecular Simulations and Design Group, Magdeburg, Germany

Rab5 is a small GTPase that serves as a membrane-associated molecular switch in early endosome fusion. Membrane anchoring is achieved via two posttranslationally attached geranylgeranyl chains at the protein C-terminus. Rab5 shuttles between the cytosol and the membrane in its inactive (GDP-bound) state, whereby solely membrane-localized active (GTP-bound) Rab5 is able to recruit effector proteins. Protein crystallography resolved the 3D structure of the catalytic G domain; however, the hypervariable N- and C-terminal regions mediating membrane association were not experimentally accessible.

Here, we present structural and dynamic properties of membrane-bound full-sequence Rab5. Models for the active and inactive states were generated by iterative structural loop refinement followed by all-atom Molecular Dynamics simulations. Rab5 associated to membranes of increasing complexity was investigated in multiple long-time MD simulations. A pure POPC bilayer as well as a simple uncharged ternary lipid mixture were found to oversimplify the plasma membrane structure and electrostatics. In contrast, a physiological six-component membrane containing the negatively charged signaling lipid PI3P allowed a detailed description of the early endosome membrane properties. Independent of the bound nucleotide our simulations revealed a high orientational flexibility of the protein. Rab5 binding to membranes is characterized by two orientation populations. On the one hand, the protein adopts a highly solvent accessible orientation perpendicular to the membrane surface. This orientation is stabilized by Rab5 association with regulatory effector proteins and preserves switch region accessibility and functionality. Moreover, we observed a tilted orientation close and almost parallel to the membrane plane. With negatively charged lipids in the membrane the protein is forced into this tilted orientation due to electrostatically favorable lipid-protein interactions. In this position, interestingly, the two switch regions mediating effector protein binding were partially buried between the protein and membrane surface. We propose that the tilted orientation may represent a reversibly formed inactive state, which can be reactivated by approaching binding partners. Thus, this behavior may allow a fast and transient deactivation mechanism on a time scale of only a few nanoseconds.