“Lipid dependent insertion of the human N-BAR domain into 2D and 3D sarcolemma model membranes”

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Abstract:

The peripheral anchorage of proteins on membranes strongly depends on the nature of the protein as well as on the membrane lipid composition. In this study, we combine structural information from 3D membrane tubulation assays with 2D Langmuir monolayer experiments to understand the insertion of the human N-BAR domain into sarcolemma membranes.

The 30 kDa N-BAR domain of Bin1 (amphiphysin 2) from skeletal muscle contributes to the generation of transverse (T)-tubules by forming protein scaffolds on the sarcolemma membrane. We show that N-BAR binding and membrane curvature generation critically depends on the lipid composition of the membrane. The role of the individual sarcolemma lipids is discussed based on binding experiments with vesicles and lipid monolayers.

The N-terminal helix of N-BAR has been proposed to promote membrane curvature generation by insertion into the outer membrane leaflet. Using Langmuir monolayer experiments, we found a pronounced incorporation of N-BAR into sarcolemma monolayers that strongly depends on the monolayer lipid composition.

Cryo electron microscopy indicates that a precise mixture of sarcolemma lipids is essential for the formation of uniform tubules with a diameter defined by the intrinsic curvature of the N-BAR domain. Cryo electron tomography with a sensitive direct electron detector revealed the supramolecular organization of the Bin1 N-BAR domain *in situ*. In contrast to the notion that N-BAR forms highly regular helical arrays on the membrane, the protein assembles into a loose helical scaffold to generate long flexible membrane tubules of defined diameter [1].

[1] B. Daum, A. Auerswald, T. Gruber, G. Hause, J. Balbach, W. Kühlbrandt, A. Meister, *J. Struct. Biol.* 194*,* **2016,** 375-382