

**Functional and Structural Studies of Insect Navpas Channel in Membrane-like Environment**

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Voltage-gated Na<sup>+</sup> (Nav) channels of insects are the target of new insecticides. Despite their great importance for agriculture, there is still a lack of information on the structure and regulation of Nav channels. Here, we studied an expression of NavPas channel from American cockroach fused with the fluorescent proteins in eukaryotic HEK cells and observed a preferential accumulation of NavPas in intracellular compartments. Only at high expression the channel was observed on the cell membrane surface. Usage of fluorescent proteins allowed us to visualize cells with surface expression of NavPas and revealed new data on its activity. The study of NavPas structure by cryo-EM in lipid-protein nanodiscs pointed on a preferred orientation of the particles making difficult to obtain a high-resolution structure. To address this issue, we collected over 23,000 images and extracted ~6 million particles. After 2D and 3D classification, 327,000 “good” particles were used for structure determination. Finally, a 2.9 Å structure was obtained for NavPas in the nanodiscs. Compared with the previously published structure in digitonin micelles, the intracellular domains were pressed to the surface of the nanodisc membrane. The obtained data provide new insights into the structure and function of the NavPas channel. The work was supported by Guangdong Province Key Special Project #2023ZDZX2072.