BAR protein Endophilin-B1 displays multiple assembly forms on artificial lipid discs

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Bin/Amphiphysin/Rvs (BAR) domain containing proteins are cytosolic, peripheral membrane proteins that regulate the curvature of membranes in eukaryotic cells. BAR protein endophilin-B1 plays a key role in fundamental cellular processes including Golgi scission, endosomal trafficking, autophagy, and apoptosis. How endophilin-B1 mediates diverse processes in the cell remains obscure and is the focus of this study.

Endophilin-B1 has an amphipathic N-terminal helix (H0), an additional amphipathic insert in helix 1 (H1i) and a C-terminal SH3 domain. It has been shown previously that endophilin-B1 can bind to and tubulate lipid vesicles *in vitro* by oligomerizing at the membrane surface and forming helical scaffolds. This tubulation activity is dependent upon H0, whose function is likely modulated by a C-terminal SH3 domain. So far structures of endophilin-B1 and other BAR proteins have been limited to either static crystal structures (not bound to a membrane), solution NMR structures of small individual domains, or low-resolution electron microscopy (EM) maps of helical scaffolds bound to tubulated vesicles.

Here we present the highest resolution cryo-EM structure of a BAR protein to date (3.47 Å) and the first structure of a BAR protein bound to nanodiscs. The data set yielded structures of endophilin-B1 in two discrete conformations. One where the dimer is only associated loosely to the membrane via N-terminal helix H0, and another, wherein amphipathic region H1i fully engages in membrane binding. The sample contained both compositionally and conformationally heterogeneous particles. Using cryoDRGN, particle species of different stoichiometries could be effectively sorted, which revealed the tremendous flexibility of post-membrane binding, pre-polymer BAR dimer organization.