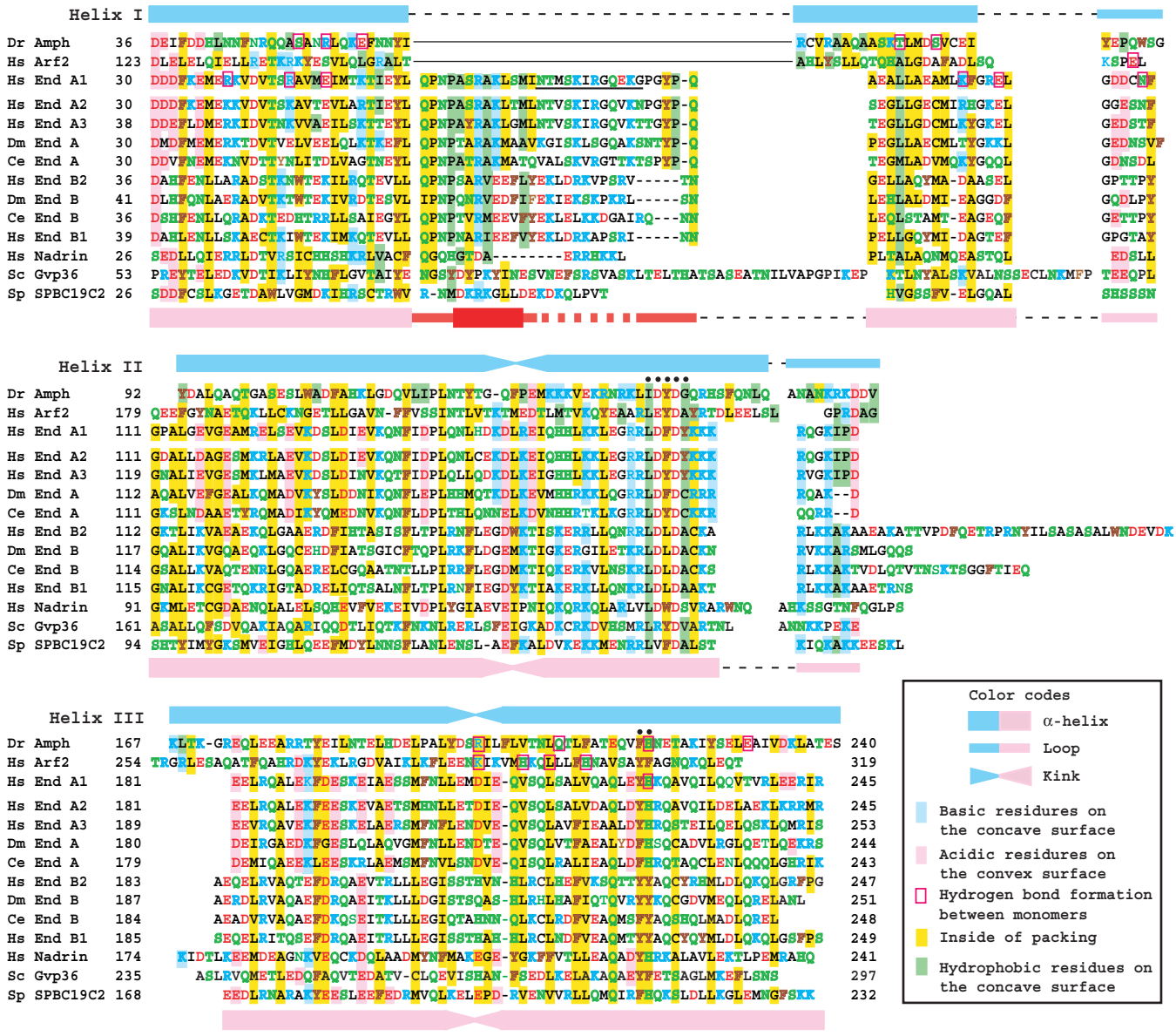
Supplementary Figure 2



Supplementary Figure 2. Structure-based sequence alignment of the BAR domains of Drosophila amphiphysin, human arfaptin2 and human endophilin-A1 (upper 3 rows). Secondary structure elements are shown at the top (Amphiphysin, blue) and the bottom (Endophilin-A1, pink and red for the endophilin-specific insertion). Residues underlined in the endophilin-A1 sequence are not seen in the crystal structure. Conserved motifs are indicated by dots. Colors of amino acid letters: basic, blue; acidic, red; aromatic, brown; alcoholic, amines and histidine, green; others, black.

Three amino acid sequences, each from three representative groups of known BAR-family proteins[1], are aligned along the spatially equivalent positions in the BAR domain structures. Total of 157 equivalent positions from helical regions are counted and only 6 (4%) and 37 (24%) of them are occupied by identical and similar residues, respectively. They are mostly hydrophobic residues involved in helix packing. Residues possibly important for the three features of BAR domains, dimerization, membrane-binding and curvature-sensing1 are not well-conserved. These are the residues connecting two monomers by hydrogen bonds (enclosed by red rectangles), basic residues

in the concave surface (highlighted by blue) for the membrane binding, and those positioned at the kinks in the helix II and III. Residues forming hydrogen bonds inside the monomer are not conserved (not shown). A dumpy shape of the End-A1-BAR dimer is due to the truncation of the helices II and III at the far ends.

Two conserved motifs predicted earlier[1] are proved to be structurally conserved: I/L-D/E-Y/F-D-G/A/Y motif in the helix II and Y/F-H/Y/F motif in the helix III. The former motif, which is positioned near the extreme ends of the dimer, displays the first, the second and the 5th residues on the concave surface. The first and the 5th residues represent only two conserved hydrophobic residues on this surface (Fig. 1A), suggesting that the motif plays an important role in the lipid binding or serves as the binding site for a yet unknown protein. The latter motif is located at the symmetric center of the dimer and thus is in close proximity to the same motif of the partner. This motif appears to act as a reference point for the dimer assembly.

The lower rows shows predicted structure-based alignment of endophilins and related proteins. Data from the secondary structure prediction by 3D-PSSM[3] are included. Human endophilin A2 (NP_003016), endophilin A3 (NP_003018), Drosophila endophilin A (NP_732383), C. elegans endophilin A (NP_491424), human endophilin B2 (NP_064530), Drosophila endophilin B (NP_725873), C. elegans endophilin B (NP_741756), human endophilin B1 (NP_057093), human nadrin (NP_060524), and putative candidates of yeast endophilins, S. cerevisiae Gvp36p (NP_012223) and S. pombe SPBC19C2 product (NP_595695).

The alignment predicts first that all these endophilin family proteins have the central appendages though the length and the sequence are diverged. The N-terminal short helix of the appendage is conserved at least in endophilins of both types. The second, acidic residues on the convex surface (highlighted by red) are conserved only in the A-type endophilins. The dipole surface potential along the convex to concave surface axis (Fig. 1A), which can facilitate proper orientation of the dimer on the negatively charged membrane, is a feature of the A-type endophilins. The third, the core structure of the dimer appears firmly conserved but the over-all shapes can alter due to the differences in the length of the appendages and of the loop localized at the extremities of the dimer, as predicted for endophilin B2.