



# Structural insights into the activation mechanism of dynamin-like EHD ATPases

Arthur Alves Melo<sup>a,b</sup>, Balachandra G. Hegde<sup>c</sup>, Claudio Shah<sup>a,b</sup>, Elin Larsson<sup>d</sup>, J. Mario Isas<sup>e</sup>, Séverine Kunz<sup>a</sup>, Richard Lundmark<sup>d,f</sup>, Ralf Langen<sup>e</sup>, and Oliver Daumke<sup>a,b,1</sup>

<sup>a</sup>Crystallography Department, Max-Delbrück-Centrum for Molecular Medicine, 13125 Berlin, Germany; <sup>b</sup>Institute of Chemistry and Biochemistry, Freie Universität Berlin, 14195 Berlin, Germany; <sup>c</sup>Department of Physics, Rani Channamma University, 591 156, Karnataka, India; <sup>d</sup>Medical Biochemistry and Biophysics, Umeå University, 901 87 Umeå, Sweden; <sup>e</sup>Zilkha Neurogenetic Institute, University of Southern California, Los Angeles, CA 90033; and <sup>f</sup>Integrative Medical Biology, Umeå University, 901 87, Umeå, Sweden

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**Eps15 (epidermal growth factor receptor pathway substrate 15)-homology domain containing proteins (EHDs) comprise a family of dynamin-related mechano-chemical ATPases involved in cellular membrane trafficking. Previous studies have revealed the structure of the EHD2 dimer, but the molecular mechanisms of membrane recruitment and assembly have remained obscure. Here, we determined the crystal structure of an amino-terminally truncated EHD4 dimer. Compared with the EHD2 structure, the helical domains are 50° rotated relative to the GTPase domain. Using electron paramagnetic spin resonance (EPR), we show that this rotation aligns the two membrane-binding regions in the helical domain toward the lipid bilayer, allowing membrane interaction. A loop rearrangement in GTPase domain creates a new interface for oligomer formation. Our results suggest that the EHD4 structure represents the active EHD conformation, whereas the EHD2 structure is autoinhibited, and reveal a complex series of domain rearrangements accompanying activation. A comparison with other peripheral membrane proteins elucidates common and specific features of this activation mechanism.**

dynamin superfamily | endocytic pathways | protein structure | membrane remodeling | autoinhibition

**E**ps15 (epidermal growth factor receptor pathway substrate 15)-homology domain containing proteins (EHDs) comprise a highly conserved eukaryotic family of dynamin-related ATPases, which regulate diverse membrane trafficking pathways (1). The single *Caenorhabditis elegans* homolog receptor-mediated endocytosis 1 (Rme-1) localizes to the endocytic recycling compartment and mediates the exit of cargo proteins to the cell surface (2, 3). Similar functions were subsequently shown for mammalian EHD1 and EHD3, which in addition control early endosome to Golgi transport (4–6). EHD4/Pincher facilitates the macroendocytic uptake of tropomyosin receptor kinase (Trk) receptors (7, 8). In contrast, EHD2 localizes to caveolae and, together with the Bin Amphiphysin Rvs (BAR)-domain containing binding partner PACSIN2 (9), stabilizes caveolae at the cell surface (10–12). Despite possessing a dynamin-related GTPase domain, EHDs bind to adenine rather than guanine nucleotides (13, 14). Similar to other dynamin superfamily members, EHD2 tubulates negatively charged liposomes and forms ring-like oligomers around the tubulated membranes (14, 15). Furthermore, the slow ATPase rate of EHD2 is stimulated by the assembly on membrane surfaces.

The crystal structure of the EHD2 dimer in the presence of the nonhydrolyzable ATP analog adenylyl imidodiphosphate (AMPPNP) revealed a dynamin-related extended GTPase domain that mediates stable dimerization via an EHD family-specific dimerization interface (14). The helical domain is composed of amino acid sequences lining the GTPase domain at its N and C terminus. We previously demonstrated by mutagenesis and electron paramagnetic spin resonance (EPR) that helix  $\alpha 9$  at the tip of the helical domain participates in membrane binding (14, 16). The Eps15-homology (EH) domains at the C terminus interact with linear peptide sequences containing Asn-Pro-Phe (NPF) motifs (17, 18). In the EHD2 dimer, they bind to an

internal Gly-Pro-Phe (GPF) motif in the linker between the helical domain and the EH domain. This interaction locks the EH domain to the opposing GTPase domain, from where it delivers its C-terminal tail into the active site (14). In this way, the EH domains were suggested to block a highly conserved second assembly site in the GTPase domain, which extends across the nucleotide binding site. GTPase domain assembly via this “G interface” is a conserved feature in the dynamin superfamily and is often accompanied by activation of the GTPase activity (19).

Our recent X-ray and EPR structural analysis demonstrated that an N-terminal stretch of 8 aa folds back into a highly conserved hydrophobic pocket of the GTPase domain in the EHD2 dimer (16). Upon its recruitment to membranes, the N-terminal residues are released and may insert into the lipid bilayer. This switch mechanism appears to negatively regulate membrane interaction of EHDs, because an EHD2 variant without the N-terminal residues showed enhanced membrane binding when overexpressed in mammalian cells. We suggested that the switch may also influence the partly disordered “KPF loop” (containing a Lys-Pro-Phe amino acid stretch) at the distal side of the GTPase domain that is required for caveolar targeting of EHD2 (10).

In this study, we elucidated the structural basis for the switch mechanism by determining the crystal structure of an N-terminally

## Significance

**Eps15 (epidermal growth factor receptor pathway substrate 15)-homology domain containing proteins (EHDs) are molecular machines that use the energy of ATP binding and ATP hydrolysis to remodel shallow membranes into highly curved membrane tubules. This activity is required in many cellular membrane trafficking pathways. In this work, we have determined a high-resolution structure of an EHD machine in the active state. The structure indicates how EHDs assemble at the membrane surface into ring-like scaffolds that deform the underlying membrane. By comparing this active state with a previously determined autoinhibited conformation, we can deduce the mechanistic details how recruitment of EHDs to membranes is regulated. A comparison with other membrane-associated molecular machines reveals commonalities and differences in the activation mechanism.**

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The authors declare no conflict of interest.

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Data deposition: The atomic coordinates and structure factors of ATPyS- and ADP-bound mouse EHD4 have been deposited in the Protein Data Bank, [www.pdb.org](http://www.pdb.org) (PDB ID codes 5MTV and 5MVF).

<sup>1</sup>To whom correspondence should be addressed. Email: [oliver.daumke@mdc-berlin.de](mailto:oliver.daumke@mdc-berlin.de).

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truncated EHD4 dimer in the oligomerized state. We reveal a complex series of domain rearrangements during activation and uncover common and specific features of this activation mechanism compared with other membrane-activated systems.

## Results

**Crystal Structure of the EHD4 Dimer.** To obtain mechanistic insights into the activation mechanism of EHD proteins, we expressed and purified an N-terminally truncated EHD4 variant (amino acids 22–541, EHD4<sup>ΔN</sup>; Fig. 1A). We reasoned that this variant may mimic the situation when EHDs are activated by membrane recruitment and release their N terminus from the GTPase domain (16). An EHD4 full-length construct was insoluble when expressed in *Escherichia coli*.

Similar to EHD2, EHD4<sup>ΔN</sup> bound to the nonhydrolyzable ATP analog adenosine 5'-(γ-thio)-triphosphate (ATPγS) with a micromolar affinity in isothermal titration calorimetry (ITC)

experiments (Fig. S1A). Furthermore, EHD4<sup>ΔN</sup> cosedimented with liposomes derived from bovine brain (Folch) lipids (Fig. S1B). Membrane binding resulted in liposome tubulation, and regular EHD4<sup>ΔN</sup> oligomers were formed at the surface of the lipid tubules (Fig. 1B and Fig. S1C). In addition, the slow ATPase activity of EHD4<sup>ΔN</sup> was 200-fold stimulated by the addition of Folch liposomes (Fig. 1C). The stimulated ATPase rate of EHD4<sup>ΔN</sup> is sevenfold higher compared with EHD2 under identical conditions (14). When C-terminally GFP-tagged EHD4 was overexpressed in HeLa cells, it localized to endosomal structures that partially overlapped with early endosomal antigen1 (EEA1) (Fig. 1D, Left and Fig. S2, see also ref. 20). The N-terminally truncated EHD4 variant showed increased membrane association (Fig. 1D, Right, quantified in Fig. S1D and E). Many membranous structures appeared tubulated in these cells, pointing to an increased membrane remodeling activity of EHD4<sup>ΔN</sup>. Taken together, these results indicate that EHD2 and EHD4 use related mechanisms of membrane recruitment and oligomerization. In particular, the N-terminal residues appear to negatively control membrane recruitment in both proteins.

For EHD4<sup>ΔN</sup>, crystals of the same space group were obtained in the presence of ATPγS and ADP and diffracted to a maximal resolution of 2.8 Å and 3.3 Å, respectively (Table S1). The structures were solved by molecular replacement and refined to  $R_{\text{work}}/R_{\text{free}}$  of 22.7%/24.3% and 20.8%/25.0%, respectively. Besides minor changes in the nucleotide binding site, both structures were essentially identical, with a rms deviation (rmsd) of 0.30 Å for all built C $\alpha$  atoms (Fig. S3A). If not otherwise mentioned, we refer in the following to the higher resolution ATPγS-bound structure.

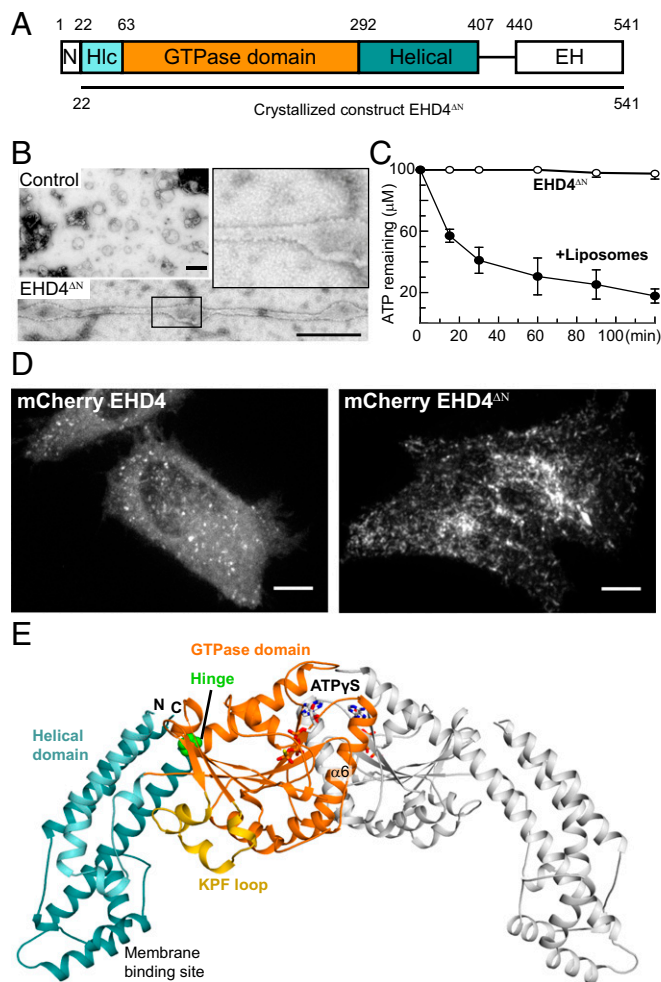
The overall architecture of EHD4<sup>ΔN</sup> is similar to that of AMPNP-bound EHD2 (Fig. 1E). For example, the GTPase domains of EHD4 and EHD2 show a highly related fold (rmsd of 0.92 Å for 194 aligned C $\alpha$  atoms; Fig. S3B). They form a dimer via an interface involving helix  $\alpha_6$ , including Arg234, Tyr236, and Trp241 (Fig. 1E and Fig. S3C). The interface is highly conserved in EHD proteins (Fig. S4) (14), but not in other dynamin-related proteins.

Electron density for ATPγS and ADP was clearly discernible in the nucleotide-binding pocket (Fig. S5A and B). Switch I in the ATPγS-bound EHD4<sup>ΔN</sup> structure adopts a different conformation compared with EHD2 (Fig. S5C). For example, the completely invariant Thr97 (Thr94 in EHD2), which coordinates the Mg<sup>2+</sup> ion in EHD2 (14) and, in analogy to dynamin (21), may position a catalytic water molecule for nucleotide hydrolysis, points away from the nucleotide in the EHD4<sup>ΔN</sup> structure. The reorientation of switch I may be caused by a newly formed contact of Arg138 from the rearranged KPF loop (see below) with the main chain of Thr96 in switch I. In the ADP-bound structure, switch I is disordered. These observations suggest an ATP-dependent conformational cross-talk between the KPF loop and the nucleotide-binding site in EHD proteins.

The EH domains were not resolved in the electron densities, despite being present in the crystallized construct. SDS/PAGE analysis of dissolved crystals showed no hints for proteolytic degradation. This observation indicates that the EH domains are disordered in the crystals and do not bind to their autoinhibitory site on top of the GTPase domains.

### A Large-Scale Rotation of the Helical Domain Promotes Activation.

The helical domain of EHD4<sup>ΔN</sup> has an almost identical architecture as its counterpart in EHD2 (rmsd of 0.67 Å for 142 C $\alpha$  atoms). However, compared with EHD2, it is rotated by ~50° relative to the GTPase domain around a hinge featuring the invariant Pro289 (Fig. 2A and Movie S1). This rotation pushes the N-terminal residues of the linker to the EH domain 12 Å away (Fig. S6A–C). In the EHD2 structure, the linker makes prominent contacts to the GTPase domain and binds via a GPF motif to the EH domain of the opposing monomer. The rotation may thus displace the linker,



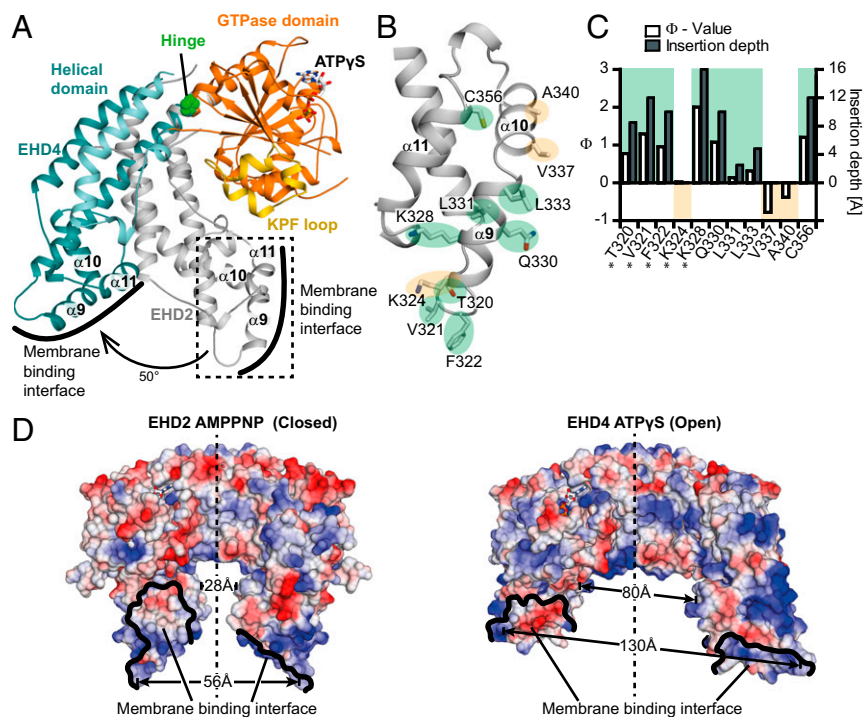
**Fig. 1.** Structure of the activated EHD4 dimer. (A) Domain architecture of EHD proteins. Numbers refer to the amino acid sequence of mouse EHD4. (B) EM micrographs of negatively stained EHD4<sup>ΔN</sup> in the presence of liposomes. As a control, liposomes were stained without the addition of EHD4<sup>ΔN</sup>. (Scale bars: B, 500 nm; D, 10 μm.) (C) ATPase assays of EHD4<sup>ΔN</sup> in the absence and presence of Folch liposomes were carried out at 30 °C. Error bars represent the range of two independent measurements. (D) EHD4 and EHD4<sup>ΔN</sup> were expressed in HeLa cells with a C-terminal mCherry tag. (E) Structure of the ATPγS-bound EHD4<sup>ΔN</sup>. In the left molecule, domains are colored according to the domain architecture, the conserved hinge around Pro289 is indicated in green. The right molecule is shown in gray. Note that the EH domains were not resolved in the electron density.

therefore releasing the EH domain from its autoinhibitory site. It also reorients the membrane binding site in  $\alpha 9$  including Thr320, Val321, Phe322, and Lys328, which, in EHD2, were shown to directly interact with the membrane (16) (Fig. 2 *A–C*).

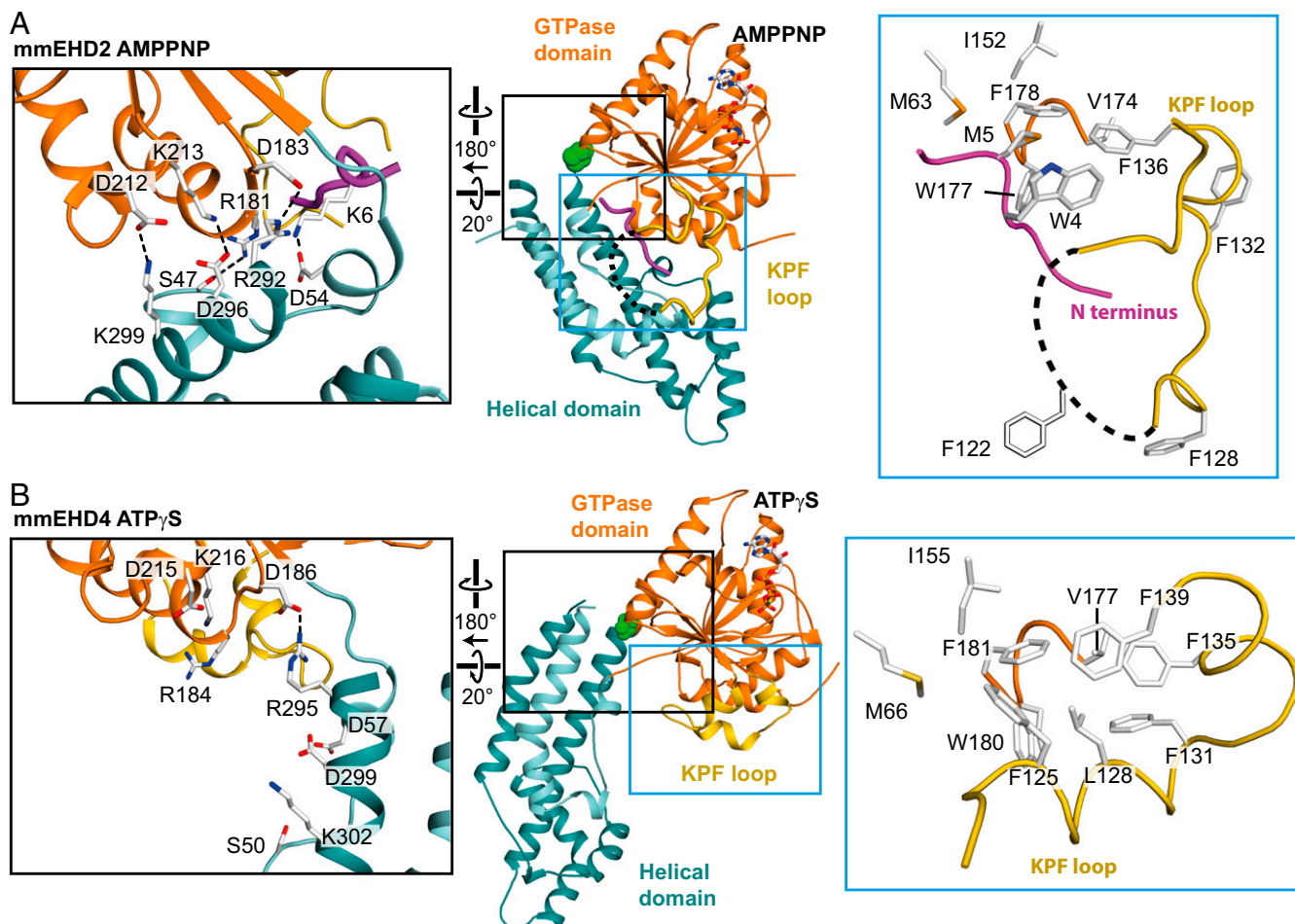
To understand the consequences for membrane binding, we used the previously established EPR assay for EHD2 as a model for the EHD family. In these experiments, a single paramagnetic spin probe is attached to a single cysteine introduced at a specific site in an otherwise cysteine-free EHD2 variant. The accessibility of the spin label toward paramagnetic spin colliders, such as oxygen and nickel ethylenediamine diacetic acid (NiEDDA), can provide information on the membrane immersion of the spin label (22). Using this assay, we now demonstrate that not only the N-terminal part of  $\alpha 9$  at the tip of the stalk, but also Gln330, Leu331, and Leu333 at the C-terminal end of  $\alpha 9$ , contribute to membrane binding (Fig. 2 *B* and *C*). Furthermore, we show that Cys356 in the adjacent helix  $\alpha 11$  directly interacts with the membrane. In contrast, Val337 and A340 in  $\alpha 10$ , which is bent away from  $\alpha 9$  and  $\alpha 11$ , did not penetrate into the membrane (Fig. 2 *B* and *C*). These results suggest that the membrane interaction site in EHD proteins extends along the parallel helices  $\alpha 9$  and  $\alpha 11$ , which, together with the entire helical domain, move en bloc during activation. In the autoinhibited EHD2 structure, the membrane binding sites from two opposing monomers point away from each other (Fig. 2*D*). In contrast, in the EHD4<sup>ΔN</sup> structure, the lipid binding regions reorient in a parallel fashion toward the membrane surface for binding. These results suggest that the EHD4<sup>ΔN</sup> structure represents a membrane-binding competent, active conformation.

**A Switch in the GTPase Domains Allows Oligomerization.** In the EHD2 structure, the helical domains form several salt bridges to the GTPase domain of the same monomer (Fig. 3*A*, black box). The corresponding salt bridges are broken in the EHD4<sup>ΔN</sup> structure (Fig. 3*B*, black box). Furthermore, we previously demonstrated that the N-terminal 8 aa in the autoinhibited EHD2 dimer fold back into a hydrophobic pocket of the GTPase domain (Fig. 3*A*) (16). In EHD4<sup>ΔN</sup>, the hydrophobic pocket in the GTPase domain is occupied by the adjacent KPF loop, which undergoes a large-scale reorientation (Fig. 3*B*, blue box, *Movie S1*). Conserved residues in this loop, such as Phe125, Leu128 and Phe131, anchor the helix into this pocket, whereas in the auto-inhibited EHD2 structure, the highly conserved Trp4 and Met5 of the N terminus (Fig. S4) occupy the equivalent space. These observations suggest that during the activation process, the KPF loop switches into the hydrophobic binding pocket of the GTPase domain.

When analyzing the crystal packing, we noticed that EHD4<sup>ΔN</sup> dimers assembled in a linear fashion in the crystals (Fig. 4*A* and Fig. S7*A* and *B*). In these oligomers, the membrane binding sites of the helical domain were oriented in the same direction, suggesting a physiologically plausible assembly. The oligomer had the same width (90 Å) as single EHD4 filaments sometimes observed on tubulated liposomes (Fig. S1*C*). Furthermore, the GTPase domains of adjacent EHD4<sup>ΔN</sup> dimers directly opposed each other via the highly conserved G interface, although they were separated by a small gap (Fig. 4*A*, *Right*). We previously proposed a similar oligomerization model for EHD2, but without the rotation of the helical domains (14).



**Fig. 2.** A rotation of the helical domains allows membrane binding. (*A*) Superposition of the GTPase domains of ATP $\gamma$ S-bound EHD4<sup>ΔN</sup> and AMPNP-bound EHD2 (PDB ID code 4CID, only the helical of EHD2 is shown). Pro289 acts as the hinge for a 50° rotation of the helical domain. (*B*) Magnification of the boxed area in *A* showing details of the membrane binding interface of EHD2. Membrane inserting (green)/noninserting (yellow) residues are highlighted. (*C*) The logarithmic ratio  $\Phi$  of the accessibilities of spin labels to the paramagnetic colliders O<sub>2</sub> and NiEDDA was calculated for EHD2 labeled at the indicated positions in the presence of Folch-SUVs (open bars referring to the left y axis). Results from residues 277, 320–324, and 328 (\*) are from ref. 16. Positive  $\Phi$  values indicate membrane insertion based on prior calibration with spin-labeled lipids. This calibration was used to convert  $\Phi$  values into membrane insertion depth of each residue (filled bars to the right y axis). (*D*) Electrostatic potentials ( $\pm 10$  kcal/mol  $\times e$ , where  $e$  is the charge of an electron) were plotted on the surfaces of the EHD2 (*Left*) and EHD4<sup>ΔN</sup> (*Right*) dimers, with the GTPase domains of both dimers in the same orientation. The distances between Phe322–Phe322 in EHD2 (56 Å), Phe325–Phe325 in EHD4 (130 Å), Pro350–Pro350 in EHD2 (28 Å), and Ala353–Ala353 in EHD4 (80 Å) are indicated. The membrane binding surface of EHD2 is more positively charged compared with EHD4, possibly reflecting different lipid binding specificities (Fig. S4).



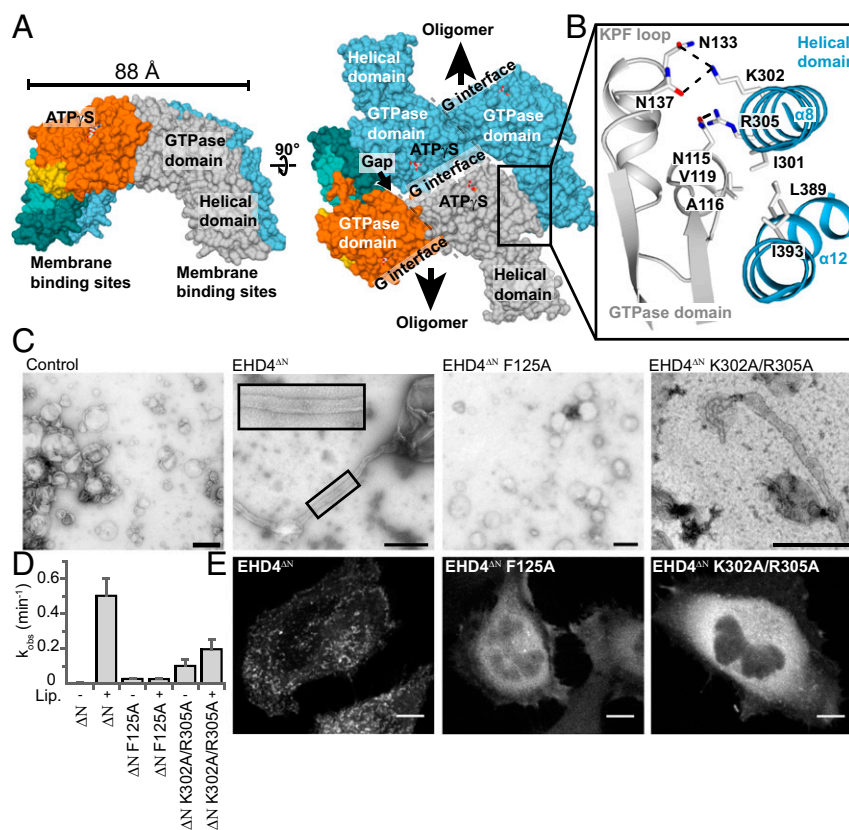
**Fig. 3.** The KPF loop undergoes a large-scale reorientation upon activation. (A) Structure of EHD2 (PDB ID code 4CID). The black box highlights the contacts of the helical domain with the GTPase domain and the N terminus in the autoinhibited state. The blue box shows the localization of the N terminus in a hydrophobic groove of the GTPase domain. (B) Structure of EHD4<sup>ΔN</sup>, with the same orientation of the GTPase domain as in A. The black box features the broken contacts between the helical domain and the GTPase domain in the active state. The blue box shows that the KPF loop occupies the hydrophobic groove in the GTPase domain, with Phe125, Leu128, and Phe131 (corresponding to Phe122, Leu125, and Phe128 in EHD2) acting as anchor points. Because of three extra amino acids in the N-terminal region of EHD4, the numbering of residues on EHD4 (residues 13–541) corresponds to the EHD2 residue number plus three (Fig. S4).

When analyzing the oligomerization determinants in these linear EHD4<sup>ΔN</sup> assemblies, we found that the rearranged KPF loop interacted via a highly conserved interface with the helical domain of the adjacent dimer (Fig. 4B and Fig. S7 B and C). To probe the involvement of this new interface for oligomerization, we aimed to disrupt it by introducing the F125A and K302A/R305A mutations in EHD4<sup>ΔN</sup> (Figs. 3 and 4B). Both mutants still bound to liposomes (Fig. S1B). Whereas the F125A completely lost its ability to remodel membranes, the K302A/R305A mutant showed reduced remodeling of membranes, as indicated by the formation of less and irregular membrane tubules (Fig. 4C). Both mutants showed slightly increased basal ATPase rates, which, however, were not or only to a minor extent stimulated by the addition of liposomes (Fig. 4D). When these N-terminal deletion variants were overexpressed in HeLa cells, they showed a severe loss of membrane recruitment (Fig. 4E and Fig. S1 D and E), and similar results were previously obtained for the F122A mutant of EHD2 (corresponding to F125A in EHD4; Fig. S4) (10). We also attempted to stabilize the oligomerization interface in full-length EHD4 by introducing the A116L and N133D mutations to enhance hydrophobic interactions and create an additional salt bridge to Lys302, respectively. When expressed in HeLa cells, the single mutants showed similar membrane recruitment compared with EHD4 (Fig. S1 D and E). Strikingly, the double mutant showed greatly enhanced membrane recruitment,

consistent with an increased oligomerization activity. Taken together, these data suggest that upon membrane recruitment and release of the N terminus, the KPF loop switches into the hydrophobic pocket of the GTPase domains and promotes oligomerization. A contact of the rearranged KPF loop with the open helical domain of the adjacent EHD dimer contributes to the regular assembly of EHDs on membranes.

## Discussion

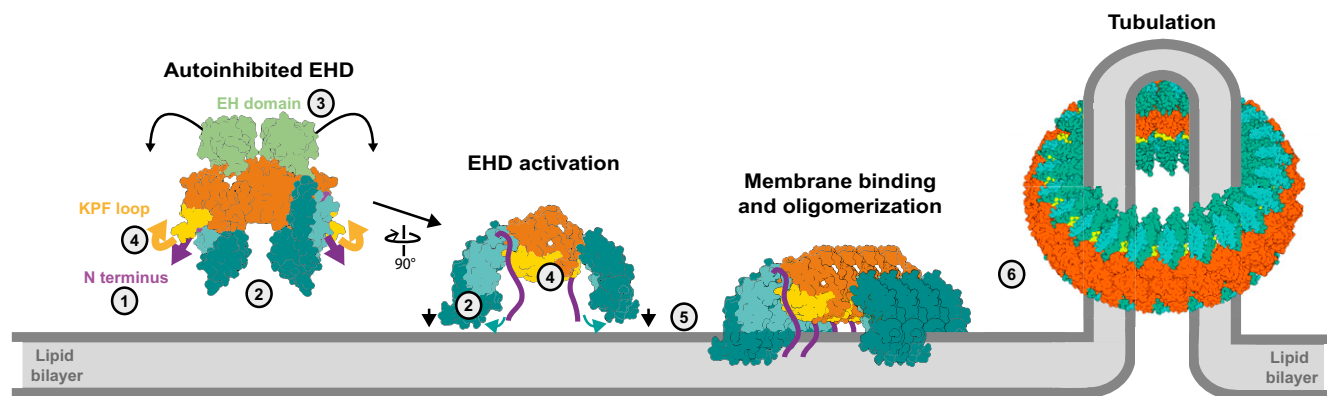
Membrane-associated assembly processes of peripheral membrane proteins are challenging to study at the structural level, and only in very few cases, high-resolution structures of such proteins in the autoinhibited and the activated, oligomerized form have been described. By removing the autoinhibitory N-terminal sequence, we obtained a membrane-binding and oligomerization-competent conformation of EHD4, which we consider as the active conformation [see also related manuscript by Hoernke et al. (23)]. In contrast, the membrane binding sites in the previous EHD2 structure were oriented away from the membrane. We therefore refer to the EHD2 structure as an autoinhibited conformation. A comparison between the active and autoinhibited form reveals a complex domain rearrangement in EHD proteins accompanying activation, resulting in an activation model for EHDs (Fig. 5). In the autoinhibited cytosolic form, the



**Fig. 4.** Oligomerization via a new interface between the KPF loop and the helical domain. (A) Two views on the EHD4<sup>ΔN</sup> oligomers in the crystals represented by two dimers. One dimer is colored as in Fig. 1E, whereas the other is colored in light blue. Note the gap between the G interfaces of assembling dimers (Right). (B) Inset shows details of the oligomerization surface involving the KPF loop and the helical domain of the opposing dimer. (C) Liposome tubulation of EHD4<sup>ΔN</sup>, EHD4<sup>ΔN</sup> F125A and EHD4<sup>ΔN</sup> K302A/R305A, as in Fig. 1B. (D) Basic and stimulated ATP hydrolysis reactions of EHD4<sup>ΔN</sup> F125A and EHD4<sup>ΔN</sup> K302A/R305A were carried out as in Fig. 1C. (E) Expression of mCherry-tagged EHD4<sup>ΔN</sup> F125A and EHD4<sup>ΔN</sup> K302A/R305A in HeLa cells, as in Fig. 1D. (Scale bars: C, 500 nm; E, 10 μm.)

N terminus is locked in the GTPase domain and the EH domains block the assembly. Upon membrane recruitment, a series of conformational changes is triggered: (i) The N terminus of EHDs is released from the GTPase domain and may switch into the membrane (see Fig. S7B for further discussion). This switch appears to activate EHDs because the EHD4<sup>ΔN</sup> construct is in the active conformation even in the absence of membranes and in both the ATP- and ADP-bound states (Fig. S3A). (ii) The helical domains rotate around the conserved Pro289. A similar

rotation mechanism was observed for dynamin and suggested to act as a power stroke (19). In contrast, the domain rotation of the helical domains in EHDs appears to regulate membrane binding by adjusting the position of the membrane-binding site in α9 and α11. (iii) Concomitant with the rotation of the helical domain, the linker to the EH domain is dislodged from the GTPase domain. Consequently, the EH domains are displaced from their autoinhibitory site on the GTPase domain, as observed in the EHD4<sup>ΔN</sup> structure. In cells, this release may be



**Fig. 5.** Activation model of EHDs. For details see Discussion; the numbers refer to release of the N terminus into the membrane (1), rotation of the helical domain (2), release of the EH domains from the autoinhibitory site (3), insertion of the KPF-loop into the hydrophobic pocket of the GTPase domain (4), membrane binding and oligomerization (5), and membrane tubulation (6).

further promoted by interactions of the EH domain with NPF motif-containing binding partners, such as syndapins/PACSINs (9, 12) and MICAL-L1 (24). (iv) Our structural analysis indicates that the KPF loop moves into the hydrophobic pocket of the GTPase domain. This loop creates a new assembly interface with the helical domain of the adjacent EHD dimer, therefore stabilizing the active conformation and promoting oligomerization of EHDs at the membrane. (v) In addition, the nucleotide-loading state of EHDs may affect the activation by nucleotide-dependent stabilization of the N-terminal loop or the KPF loop in the GTPase domain. Supporting this hypothesis, the KPF loop and switch I interact in an ATP-dependent manner. Furthermore, the removal of the EH domain tail from the active site may allow the ATP-dependent oligomerization of EHDs in ring-like structures via the conserved G-interface, which could explain the strict ATP dependency of assembly (Movie S2) (16). Such assembly would facilitate (vi) a direct coupling of EHD oligomerization with the creation of membrane curvature.

Whereas the overall architecture of EHDs is related to dynamin, the oligomerization modes of these proteins differ fundamentally: Oligomerization of dynamin/MxA/DNM1L in helical filaments is mediated by three assembly interfaces in the helical stalk (19). The GTPase domains contribute to assembly by mediating GTPase-dependent contacts between adjacent filaments. In this way, nucleotide binding and hydrolysis can induce the rearrangement of adjacent filaments assembled via the stalk, leading to constriction of the underlying membrane. In contrast, EHDs use a unique interface in the GTPase domain for dimerization and use the ATP-dependent G interface for further oligomerization. Contacts between the GTPase domain and the helical domain of the next dimer contribute to oligomer formation. The architecture of the EHD oligomers excludes a nucleotide-driven sliding mechanism because nucleotide-dependent contacts are formed within and not between adjacent filaments. However, the assembly mode is well suited for the stabilization of membrane curvature by oligomerizing into ring-like tubular oligomers.

Despite the involvement of different domains, there are striking parallels in the activation mechanisms of EHDs and dynamin (Fig. S8). In the autoinhibited dynamin tetramer, an intramolecular contact of the pleckstrin homology (PH) domain with the oligomerization surface of the stalk prevents the assembly in the cytosol (25), whereas an autoinhibitory contact of the C-terminal EH domain with the GTPase domains takes over this function in EHDs. Such intramolecular inhibitory contacts are also observed in other peripheral or integral membrane proteins, such as BAR-domain containing proteins (26, 27), SNAREs (28), ESCRT-III (29), or the WASP protein (30) (Fig. S8 B–E). Binding of specific elements to membranes or interaction partners shifts the equilibrium toward the active state. The formation of new interactions in the membrane-bound oligomer is accompanied by the loss of intramolecular interactions in the autoinhibited dimer. Such mechanism may allow reversibility of membrane recruitment and oligomerization.

Taken together, our study elucidates a complex activation mechanism of EHD family proteins, revealing common and specific features how peripheral membrane proteins scaffold cellular membranes.

## Materials and Methods

Protein expression and purification were carried out as described before (14). A detailed description of the purification, crystallization, structure solution, refinement, biochemical experiments, electron microscopy, cell biology and microscopy analysis can be found in *SI Materials and Methods*.

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