SPFH protein cage - one ring to rule them all

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This is the final version of the accepted manuscript. The original article has been published in final edited form in:

Cell Research
2022 FEB ; VOL(ISS): PAGE-PAGE
2021 DEC 21 (first published online: final version)
doi: 10.1038/s41422-021-00605-7

URL: https://www.nature.com/articles/s41422-021-00605-7

Publisher: Nature America (Springer Nature)

Structure of the SPFH protein cage revealed - One Ring to rule them all

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Members of the SPFH protein family comprise a universally conserved family of membrane-associated protein scaffolds that compartmentalize membranes, but their detailed molecular mechanism has remained unclear. In the current issue of Cell Research, Ma et al. determined the cryoEM structure of the bacterial SPFH heterodimer HflK/C, which assembles into a giant molecular cage encasing four AAA+ protease hexamers [1]**. The structural work rationalizes how SPFH proteins confine their client proteins in specialized membrane microdomains.**

The Stomatin/prohibitin/flotillin/HflKC (SPFH) protein family is an ancient and conserved family of proteins that share highly conserved SPFH1 and 2 domains [2,3]. Many functional studies in bacteria, worms, and mice have shown critical functions for these proteins in diverse physiological processes ranging from mitochondrial function to the regulation of mechanosensitive ion channels required for touch sensation in worms and mammals [4,5]. The importance of this family of proteins for disease pathology has been well documented and there are now several examples of small drug like molecules developed to target these proteins and treat pathologies ranging from neuropathic pain to cancer [6,7]. It appears that SPFH domain proteins display two common conserved features, first they are oligomeric and second, they are often localized to cholesterol rich microdomains in membranes. Despite the hugely diverse range of functions shown by SPFH proteins across the animal kingdom, it is plausible that a core structural principle underlies their function. In the highlighted paper from Ma and colleagues, such a core structural principle may indeed have emerged.

Ma et al. expressed and purified detergent-solubilized oligomers of the *E. coli* SPFH proteins HflK/C in complex with FtsH, the client AAA+ protease [1]; a remarkable achievement given that all three components are integral transmembrane (TM) proteins. Using extensive particle sorting, they eventually obtained a 3.3 Å resolution cryoEM density for an HflK/C 24-mer bound to four FtsH hexamers.

While the cytoplasmic portions of HflK and FtsH, including the AAA+ domains, were mostly disordered, the periplasmic regions of all three components and the upper part of the TM regions of

HflK and HflC could be modelled (Fig. 1a, b). HflK/C consist of an N-terminal TM domain, followed by an SPFH1 and SPFH2 domain, a long coiled coil (CC1), a shorter coiled coil (CC2) and a C-terminal region. In the complex, domains of alternate HflK/C units assemble in parallel to build a spectacular ring-shaped closed cage containing 12 copies of each subunit. While the SPFH1 and 2 domains form the shoulder of the cage, the CC1 domains build the cap, and CC2 and the C-terminal region the lid of the cap. The identified interaction interfaces appear at least to be partially conserved in SPFH domain proteins, although the subunit stoichiometry appears to vary. While prohibitins oligomerize into ring-shaped heteromeric complexes with a similar mass to HflK/C, the related major vault protein forms an even larger cytoplasmic assembly with a 39-fold rotational symmetry (Fig. 1c, top). Recently, the FliL protein from the marine bacterium *Vibrio alginolyticus* was shown to contain one SPFH2 domain and, as part of the flagellar motor, assembles into a 10-fold symmetric ring (Fig. 1c, bottom). Thus, depending on the specific cellular function, SPFH proteins can form rings of different sizes. Previously reported assemblies of SPFH domain proteins in mammals and archaebacteria revealed dimers or trimers, respectively [2,3]. These assemblies of truncated proteins may still be functionally meaningful, but could also result from artificial interfaces not normally exposed in the cellular or membrane environment.

The periplasmic N-terminal domains of FtsH formed four hexamers, whose structures were previously described [8]. The current structure revealed interaction sites in the cage provided by the linker located in between the SPFH1 and SPHF2 domains of HflK. Interestingly, the C-terminal region of HflK extends into the HflK/C cage and contributes to the interaction with FtsH. Membrane integration of the cage is mediated by the TM domains which encompass a circular/quadratic membrane area with a diameter of about 16 nm. It is intriguing that the AAA+ protease domains of HflK/C are actually localized on the opposite site of the membrane to the SPFH cage, and a similar topology is envisaged for the prohibitins and one of their client mitochondrial AAA+ proteases [5].

Substrates of the encaged AAA+ proteases are mostly TM proteins so that the SPFH cage could potentially control substrate access from both sides of the membrane.

A corral is a small enclosure used to confine horses, and perhaps we can now think of the SPFH cage as corral to confine and regulate membrane proteins. In this work, the authors show how the SPFH1 domains appear to insert stretches of hydrophobic residues into the membrane, which could induce membrane buckling or spontaneous membrane tension in one of the membrane leaflets. SPFH domain proteins are often lipid-modified at residues close to the membrane and so the SPFH domain cage potentially creates a specific lipid environment of defined size. SPFH proteins like MEC-2 in worms and STOML3 in mammals are important regulators of mechanosensitive channels [9,10]. Lipid composition and membrane tension are both important determinants of mechano-gating of a variety of molecularly distinct channel entities. It is conceivable that mechanically gated channels like the Deg/ENaC proteins MEC4/MEC10 and some acid-sensing ion channel family members (e.g. ASIC3) or PIEZO2 trimers are most efficiently gated when confined to a patch of lipids kept under tension within an SPFH cage. Indeed, super resolution dSTORM imaging of STOML3 in the plasma membrane shows it clustering in membrane domains with dimensions consistent with those of the HflK/C cage [7]. In terms of membrane footprint, an SPFH cage with dimensions similar to that of HflK/C could in theory accommodate one PIEZO trimer or 3-4 trimeric channels of the Deg/ENaC family (Fig. 1d). The present study provides a high-resolution view of one SPFH cage, but other SPFH domain proteins appear to also form rings with different diameters or cap configurations [5,11].

The structure of the SPFH domain cage provides a solid basis to study the dynamics and regulation of cage assembly and disassembly. Furthermore, small drug-like molecules may stabilize or destabilize SPFH cages, and such high-resolution structures could form the basis of a more rationale and targeted drug design. Thus, SPFH cages of varying diameters and conformations could be universal modules to regulate membrane proteins involved in cellular processes important for a huge variety of human diseases.

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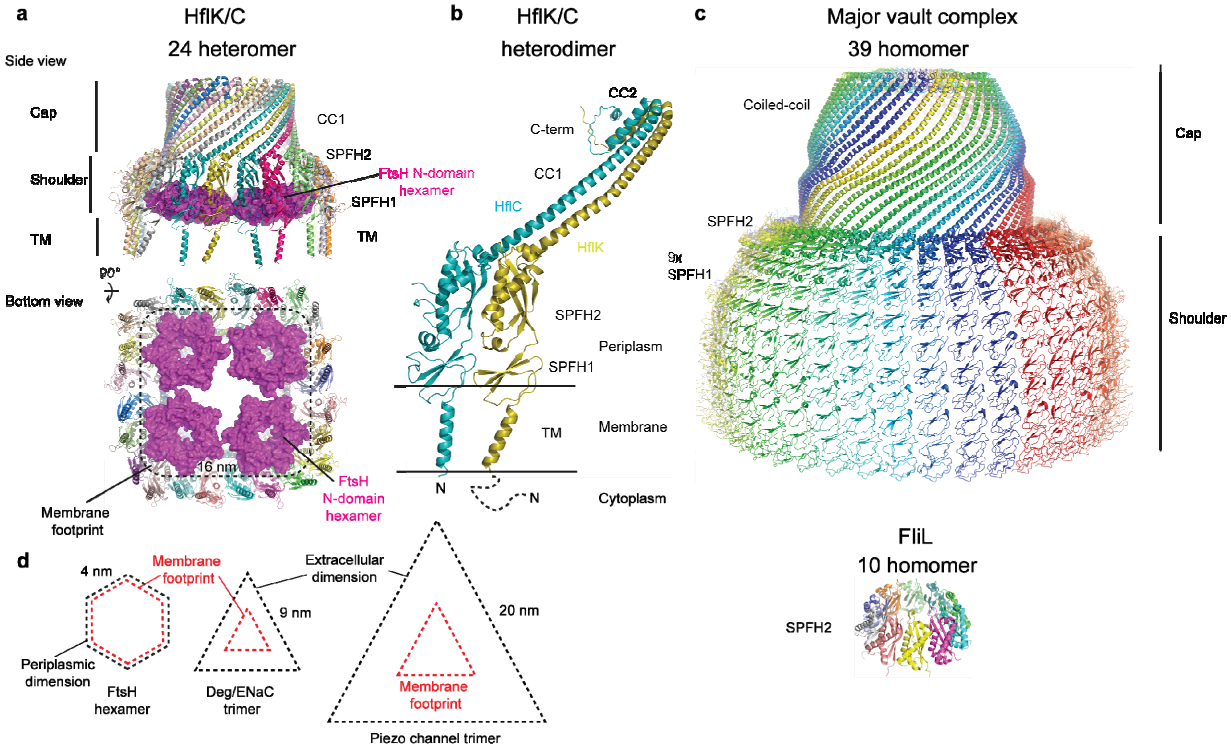

Figures 1: SPFH protein cages and their substrates. a) Top and bottom view of the HflK/C 24 heteromer, with four encased FtsH N-domain hexamers shown in surface representation (magenta). The membrane footprint, indicated as dashed box, has an approximate diameter of 16 nm. **b**) Close-up view on the HflK/C hetero-dimer, showing the domain architecture. The deduced position of the membrane is indicated. **c**) Comparison to other SPFH domain oligomers. (top) The major vault complex (pdb 4V60) with a 39-fold symmetry. Similar to HflK/C, the SPFH1, SPFH2 and coiled coil domains form a cage-like structure with a cap and a shoulder. In the cytosol, two of these oligomers further assemble to build a closed encasing. (bottom) The SPFH2 domains of FliL (pdb 6AHQ) form a homo-decameric oligomer, using a similar assembly mode as that of HflK/C and the major vault complex. **d**) shows the rough dimension of SPFH domain client proteins in the periplasmic/extracellular space (black dashed lines) and at the membrane (red dashed lines). The structures in **a**, **c** and **d** are roughly drawn to scale.