

# ESCRT Filaments as Spiral Springs

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In a recent issue of *Cell*, Chiaruttini et al. (2015) reveal the mechanical properties of the mysterious spiral filaments formed by the yeast ESCRT-III protein Snf7. The spirals are shown to be springs whose bending drives membrane deformation and perhaps membrane scission.

Eukaryotic cells have evolved several protein machineries to catalyze and regulate membrane vesicle budding. When budding takes place toward the cytoplasm, it is most often mediated by vesicle coats such as clathrin, COPI, or COPII. But whenever membrane budding takes place with the opposite topology, away from the cytoplasm, the key players are the endosomal sorting complexes required for transport (ESCRTs) (Hurley, 2015). The ESCRT machinery was first studied in the context of the budding of membrane proteins into the interior of endosomes on their way to destruction but has since been implicated in a multitude of membrane budding events. Such budding events include microvesicle release, plasma membrane wound healing, release of HIV and other viruses, and daughter cell abscission (Hurley, 2015). In the Crenarchaea, ESCRT homologs serve as the membrane abscission machinery in cell division, which thus appears to be their most ancient function. The most conserved and fundamental function of the ESCRTs is thus the severing of narrow membrane necks from their inner surface (McCullough et al., 2013). In a recent paper in *Cell*, Chiaruttini et al. (2015) now show that a filament consisting of one ESCRT-III subunit, Snf7, has special physical properties that help explain its unique biological activities.

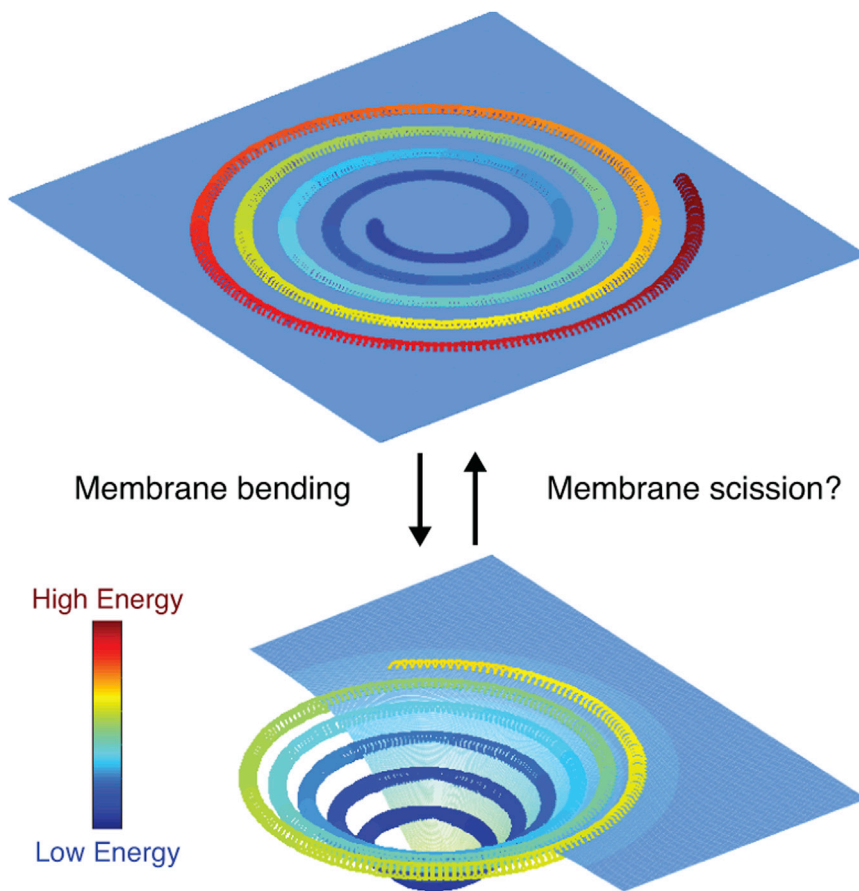
The many ESCRT proteins function in diverse budding events on a wide range of size scales. Upstream ESCRTs in various constellations recruit the membrane-cutting ESCRT-III proteins. ESCRT-III proteins are 25 kDa  $\alpha$ -helical proteins that exist as autoinhibited monomers in the cytoplasm. When upstream ESCRT factors recruit them to their site of action, they polymerize locally on the

membrane. Depending on the type of membrane-severing event, different subsets of ESCRT-III proteins are recruited. The ESCRT-III protein Snf7 participates in all known ESCRT-mediated scission reactions, contains a unique N-terminal hydrophobic sequence that drives its tight membrane interaction (Buchkovich et al., 2013), and is the most abundant protein in ESCRT-III assemblies (Teis et al., 2008). Overexpression of Snf7 leads to appearance of tubular invaginations (Hanson et al., 2008). These structures provided the first direct evidence that Snf7 could deform membranes, but their precise connection to scission has been unclear. Despite the central role of membrane-associated Snf7 filaments for all ESCRT-mediated processes, the physics of their assembly is not well understood. What can be said is that Snf7 (and, in general, ESCRT-III) filaments differ in many ways from the well-known F-actin and microtubule protein filaments. For example, ESCRT-III subunits do not have the nucleotide-hydrolyzing activity of actin and tubulin. Instead, their assembly is driven by the release of autoinhibition by membranes or upstream ESCRTs. However, in one parallel to microtubules, the disassembly of ESCRT-III is carried out by the AAA<sup>+</sup> ATPase Vps4, which is closely related to the microtubule-severing enzyme spastin.

High-speed atomic force microscopy (HS-AFM) (Ando et al., 2013) is the main enabling technology for the new advances (Chiaruttini et al., 2015). These devices have the potential to revolutionize our understanding of protein self-assembly on membranes by providing single-protein structural resolution in real time. Chiaruttini et al. (2015) have now begun

to make this potential real. First, the authors analyzed nanoscale patches of Snf7 on flat membranes. The authors discovered that the patches were formed from spiral filaments similar to ones observed previously by EM (Hanson et al., 2008; Shen et al., 2014). Surprisingly, the authors found an apparent nucleation ring—a single closed ring of roughly 25 nm diameter—that forms spontaneously on membranes and must be broken to induce polymerization of a spiral filament. To verify that this nucleation ring is a preferred state of Snf7 polymers, the authors use nano-dissection with the AFM tip to disrupt large spirals and verify that broken polymers spontaneously reform into smaller rings. This suggests a preferred diameter of 25 nm for Snf7. In addition, through mathematical modeling, the authors determine a persistence length of 260 nm for Snf7 polymers. The persistence length of a polymer is a measure of its stiffness. For comparison, DNA has a persistence length of 50 nm and is often found wrapped around  $\sim$ 8-nm-diameter nucleosomes, while actin and microtubules are much stiffer, with persistence lengths of 15  $\mu$ m and 6  $\mu$ m, respectively.

Because Snf7 filaments form spirals outward from the nucleation ring, the filaments must “un-bend,” relative to their preferred curvature, to form the larger rings of the spiral. In so “un-bending,” they accumulate elastic energy of deformation. This leads the authors to propose that Snf7 deforms membranes as follows. Snf7 spirals polymerize on flat membranes, growing beyond their preferred 25 nm curvature. This energy is eventually released through spiral expansion and inward buckling of the



**Figure 1. Energy Storage and Release by the Snf7 Spiral Spring**

Spiral springs grow inward to outward, with the energy of deformation (color coded from blue to red) increasing as the outer coils increasingly depart from the preferred diameter of 25 nm. In the model proposed by Chiaruttini et al. (2015), the energy stored in the spiral exceeds the energetic cost of bending the membrane, and elastic energy is transferred from the spiral to the membrane (downward arrow, left; notice the color change in the bent portion of the membrane). In an alternative scenario, the membrane might already be deformed prior to the arrival of Snf7, perhaps by formation of a viral bud or by upstream components of the ESCRTs. In this model (upward arrow, right), the elastic energy of the membrane might exceed the cost of energy of the flat spiral, particularly if late-acting downstream ESCRT subunits lowered the energy of the outer coils. Flattening of the helical coil would buckle the membrane, leading to scission.

interior rings, causing the underlying membrane to buckle and bend underneath the filaments (Figure 1). The energy of spiral deformation is thus released and converted into energy of

membrane deformation. The authors verify through mathematical modeling that the energy contained in these spirals is sufficient to deform the membrane. Through AFM, they directly visu-

alized that the innermost rings buckle into the membrane.

All in all, Chiaruttini et al. (2015) convincingly show that Snf7 filaments are spiral springs. These data explain how a flat Snf7 spiral could deform into a tubular evagination of the type that has been seen in overexpression systems (Hanson et al., 2008). However, the fundamental activity of the ESCRTs, and the one whose mechanism is most eagerly sought, is membrane scission. Run in reverse, the spiral spring would seem to be the ideal means to drive the buckling, and thence scission, of the neck of a loaded vesicle. It would be interesting if it turned out that the most important insight from the observations of Chiaruttini et al. (2015) was the implications for scission, rather than tubulation. The HS-AFM methodology, extended to more complex and realistic systems, including subunits other than just Snf7, will likely continue to have a key role in elucidating scission.

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