

## Size Matters: Molecular Architecture of TPPII and its Impact on Proteolytic Activity

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Cytosolic protein degradation proceeds largely via large, self-compartmentalized protein complexes containing their active sites in a secluded compartment. The paradigm for such a complex is the 26S proteasome, which degrades ubiquitinated proteins in an ATP-dependent manner [1]. In the successive degradation of the resulting, relatively small products also large complexes are involved. One of them is Tripeptidyl Peptidase II (TPPII), an aminopeptidase of the subtilisin-type of serine proteases, which has a mass of several MDa [2; 3]. Owing to its extraordinary size, its apparent potential to substitute for some of the proteasome's functions as well as to its implication in MHC-class I peptide trimming, in neuropeptide degradation, in apoptosis and in sepsis, the TPPII complex is attracting increasing attention [4].

Using cryo-electron microscopy and single-particle reconstruction we have shown that the 150 kDa subunits of *Drosophila* TPPII assemble into a spindle-shaped 6 MDa complex consisting of two twisted strands [5]. Each of the strands comprises ten stacked dimers, which display two-fold symmetry and are interdigitated. This superstructure is stabilized by a "double-clamp" at the spindle poles where the terminal dimer of one strand locks the two terminal dimers of its neighboring strand and vice versa [6]. Compared to ATP-dependent proteolytic systems like the 26S proteasome, where the linear assembly of the unfoldase- and protease-subcomplex reflects the sequence of steps necessary for substrate degradation, the functional reason for the peculiar architecture of TPPII is not as obvious.

For human TPPII it has been reported that only the assembled complex is fully active and that dissociation into dimers leads to activity loss. Also in *Drosophila* TPPII the dimer possesses only approx. 1/10th of the activity of the fully assembled complex. When studying the assembly of the heterologously expressed complex in *E. coli* cell extracts, we found, that in the course of strand elongation the specific activity increases with strand length and correlates with the number of inter-dimer contacts formed. Accordingly, long single strands possess the same specific activity as spindles consisting of two strands but the spindle complexes are the thermodynamically stable form as shown by treatment with destabilizing agents, which led to dissociation of single strands but left spindles intact. Whether there is more to the spindle shape of TPPII than providing high activity at high stability is an open question [7].

Electron microscopical studies of a TPPII variant with an N-terminal bulky tag allowed us to map the N-terminal domains, which include the catalytic residues, to the inner backbone of the TPPII strands. At the outer surface of the strands the linear stacking of the dimers leads to the formation of a channel or an arcade with lateral openings through which substrate flow might occur [6]. Since there is no obvious reason for channeling the relatively small substrates of TPPII longitudinally through the strands, we propose that the arcade enables a lateral substrate-flow leading to the saturation of all active sites. At the same time it might protect the substrates from complete hydrolysis in the cytosol, which is a feature required for the role of human TPPII in peptide trimming. Thus, our current work is directed to the identification of suitable substrates for labeling and visualization within the TPPII complex via cryo-electron microscopy.

## References

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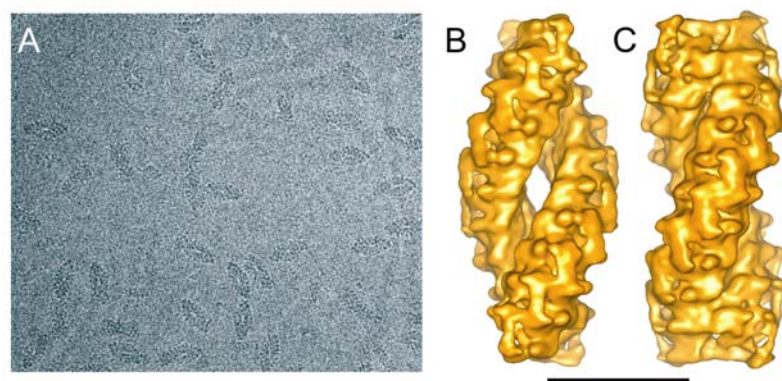


Fig. 1: Electron microscopy of Tripeptidyl Peptidase II. A. TPP II particles embedded in vitreous ice. B,C. 3D reconstruction of TPPII at a resolution of 2.2 nm. Two views of the complex are shown from two perpendicular directions. Scale bar = 30 nm.