Structure of the Native Doublet Microtubule from *Trichomonas* vaginalis Reveals Parasite-specific Proteins as Potential Drug Targets

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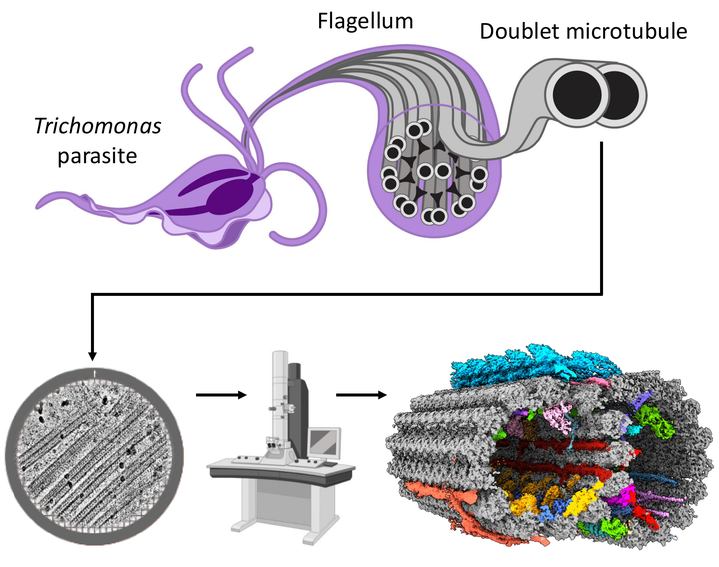
Abstract

Doublet microtubules (DMTs) are flagellar components required for the parasite *Trichomonas vaginalis* (*Tv*) to swim through the human genitourinary tract and cause trichomoniasis, the most common non-viral sexually transmitted disease. The lack of high resolution DMT structures has prevented structure-guided drug design to manage *Tv* infection. Here, we determined the cryo-electron microscopy structure of native Tv DMTs, identifying 29 unique proteins, including 18 microtubule inner proteins and 9 microtubule outer proteins. Notably, the parasite-specific proteins *Tv*FAP35 and *Tv*FAP40 form filaments at the DMT junctions, providing structural stability important for *Tv* locomotion. Additionally, *Tv*FAP40 has a small molecule coordinated within a charged binding pocket, which may be targeted by an inhibitor. These structural findings shed light on the diversity of flagellar adaptations and provide a framework to inform rational design of therapeutics.



FAP35 is a Key Player in Outer Junction Stability and Organization

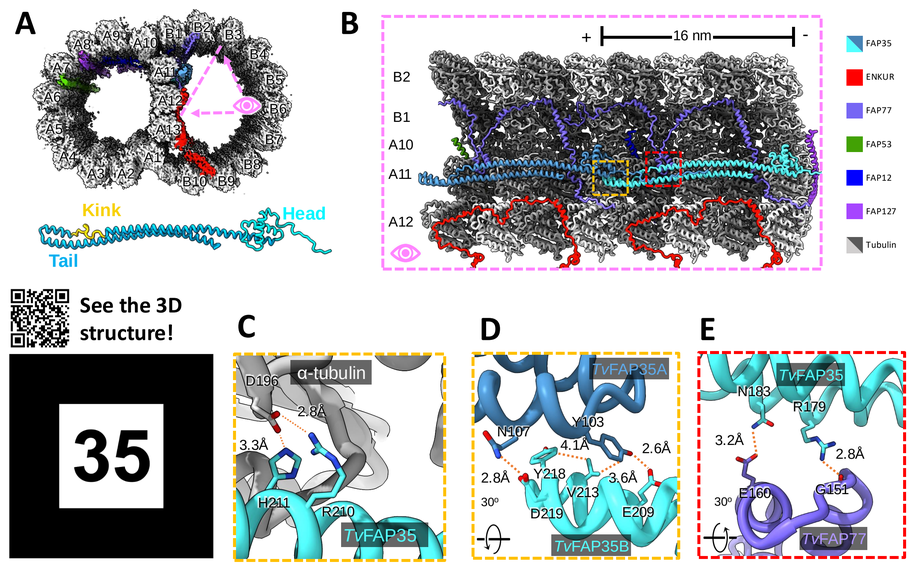
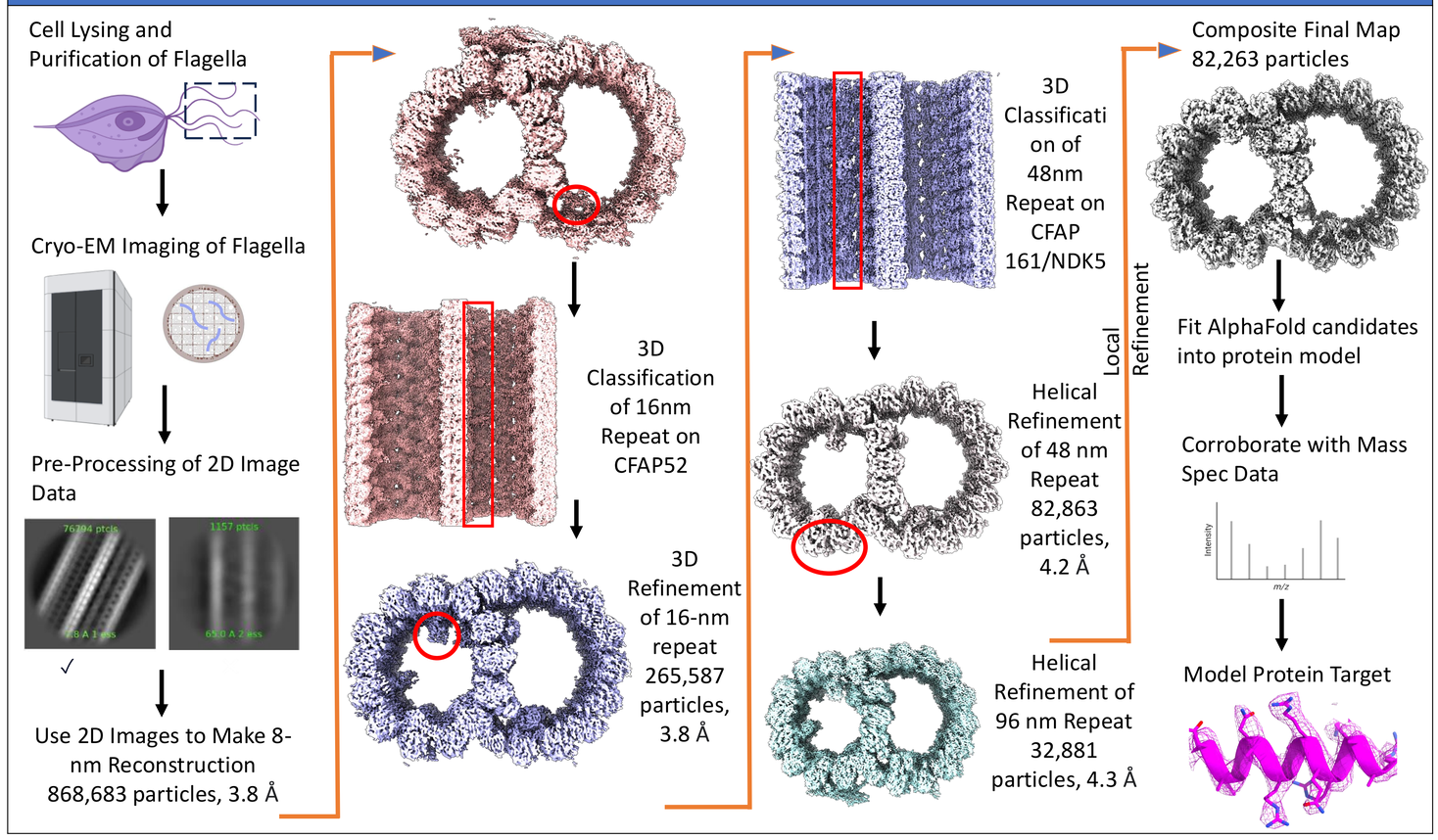


Fig 2A, Sagittal cross-section of microtubule doublet focused on the outer junction; **Fig 2B**, model view of Fig2A cross section with FAP35 at the outer junction, **Fig2C**, interactions of FAP35 with tubulin protofilament; **Fig2D,** electrostatic and hydrophobic interactions that facilitate end to end association of FAP35; **Fig2E,** FAP35 and FAP77 association via hydrogen bonding.

Cryo-Electron Microscopy Allows Visualization of Outer Microtubule Doublets at a High Resolution



FAP40 Restructures the Inner Junction of Microtubule Doublet and is Structurally Similar to Other Uncharacterized Proteins in Pathogenic Protists

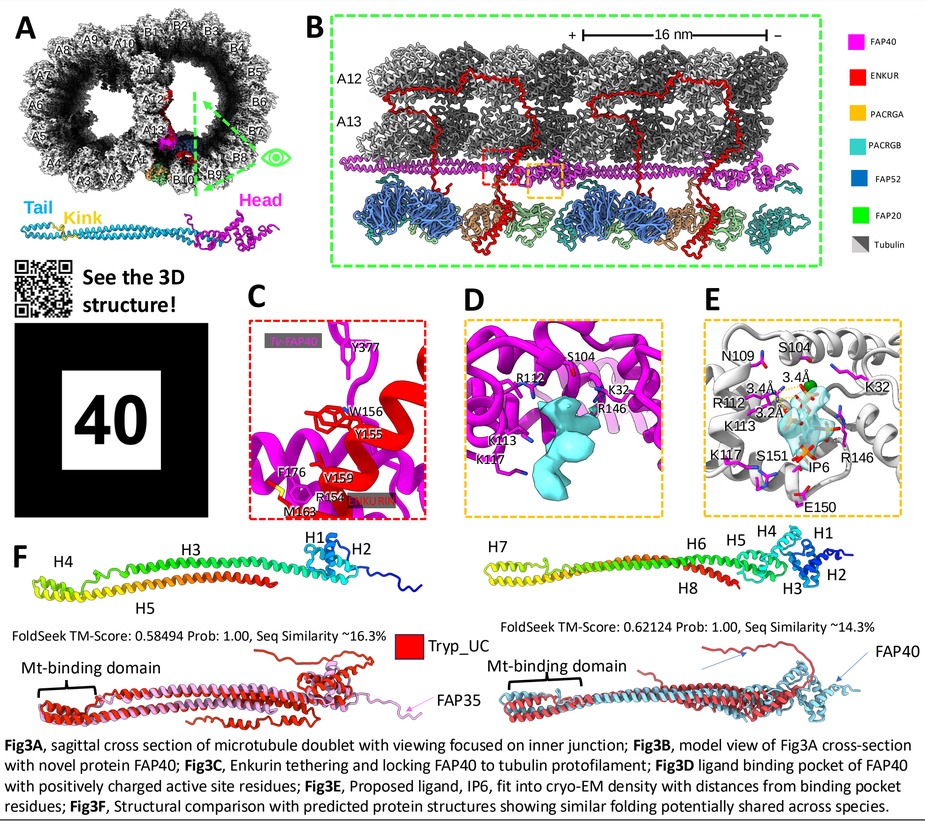
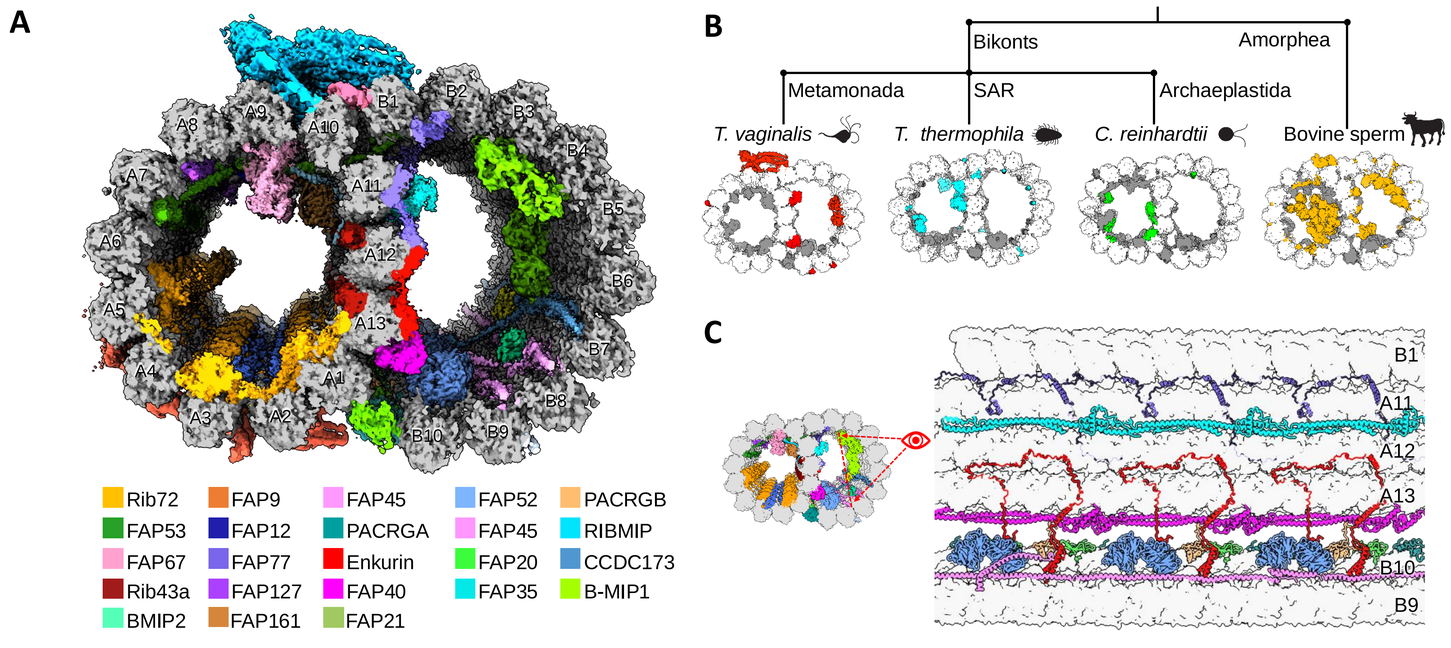


Fig3A, sagittal cross section of microtubule doublet with viewing focused on inner junction; **Fig3B**, model view of Fig3A cross-section with novel protein FAP40; **Fig3C**, Enkurin tethering and locking FAP40 to tubulin protofilament; **Fig3D** ligand binding pocket of FAP40 with positively charged active site residues; **Fig3E**, Proposed ligand, IP6, fit into cryo-EM density with distances from binding pocket residues; **Fig3F**, Structural comparison with predicted protein structures showing similar folding potentially shared across species.

Single Particle Analysis Reveals Microtubule Inner Proteins Integral to Parasite Motility



*FAP > Flagellar Associated Protein *UD > Unidentified *DUF > Domain of Unknown Function *CCDC > Coiled-Coil Domain Containing Protein *Rib > Ribbon Associating MIP

Fig 1A, Front View of 48-nm repeating doublet microtubule, highlighting 19 conserved and novel MIPs; **Fig1B**, Phylogeny tree of example organisms with comparisons of their DMTs (bottom) with tubulin (white), conserved flagellar associated proteins (FAPs) (grey), and species-specific FAPs (colored); **Fig1C**, Cross-section through DMT with view orthogonal to filament axis, highlighting B-tubule ribbon proteins.

Summary

- High-resolution 3.6 Å Reconstruction of *Tv* Microtubule Doublets
- Characterization of Novel Proteins FAP35 and FAP40
- *Tv*-specific adaptations to stabilize outer and inner junction

References and Acknowledgements

1. World Health Organization. (2023, July 10). Sexually Transmitted Infections (STIs).
2. Kusdian G, Gould SB. The biology of Trichomonas vaginalis in the light of urogenital tract infection. Mol Biochem Parasitol. 2014 Dec;198(2):92-9. doi: 10.1016/j.molbiopara.2015.01.004.
3. Benchimol M. Trichomonads under Microscopy. Microsc Microanal. 2004 Oct;10(5):528-50. doi: 10.1017/S1431927604040905.
4. Nia, Shervin. (2023). bioviewer.net (v1.0) [Source code]. Zenodo. doi: 10.5281/zenodo.10152797

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