CRYO-ELECTRON MICROSCOPY CHARACTERIZATION OF FLAGELLAR MICROTUBULE DOUBLETS IN PATHOGENIC TRICHOMONAS VAGINALIS

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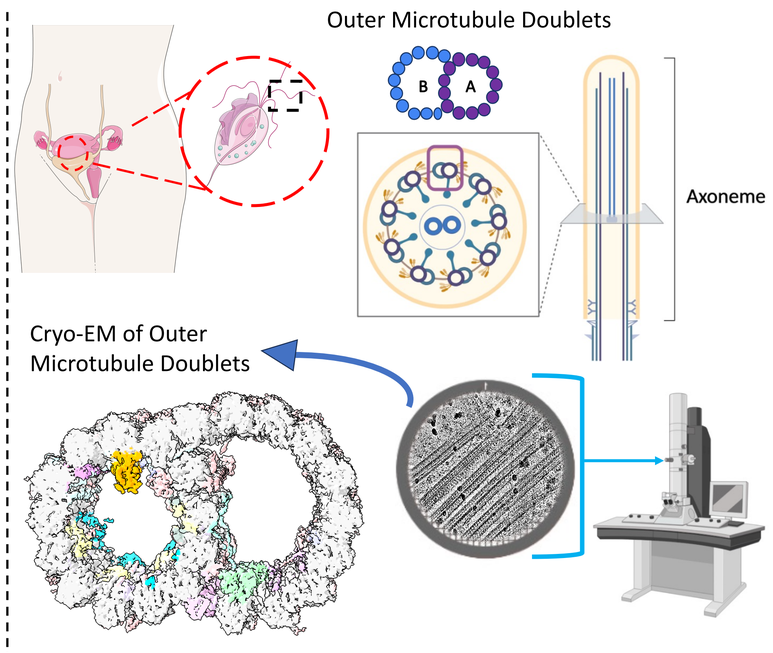
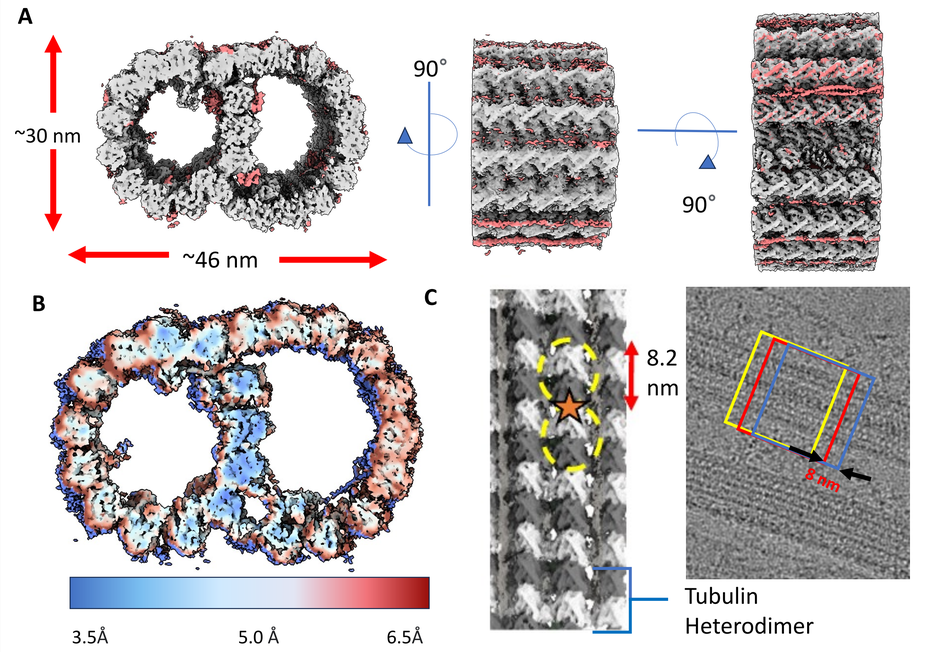
Abstract

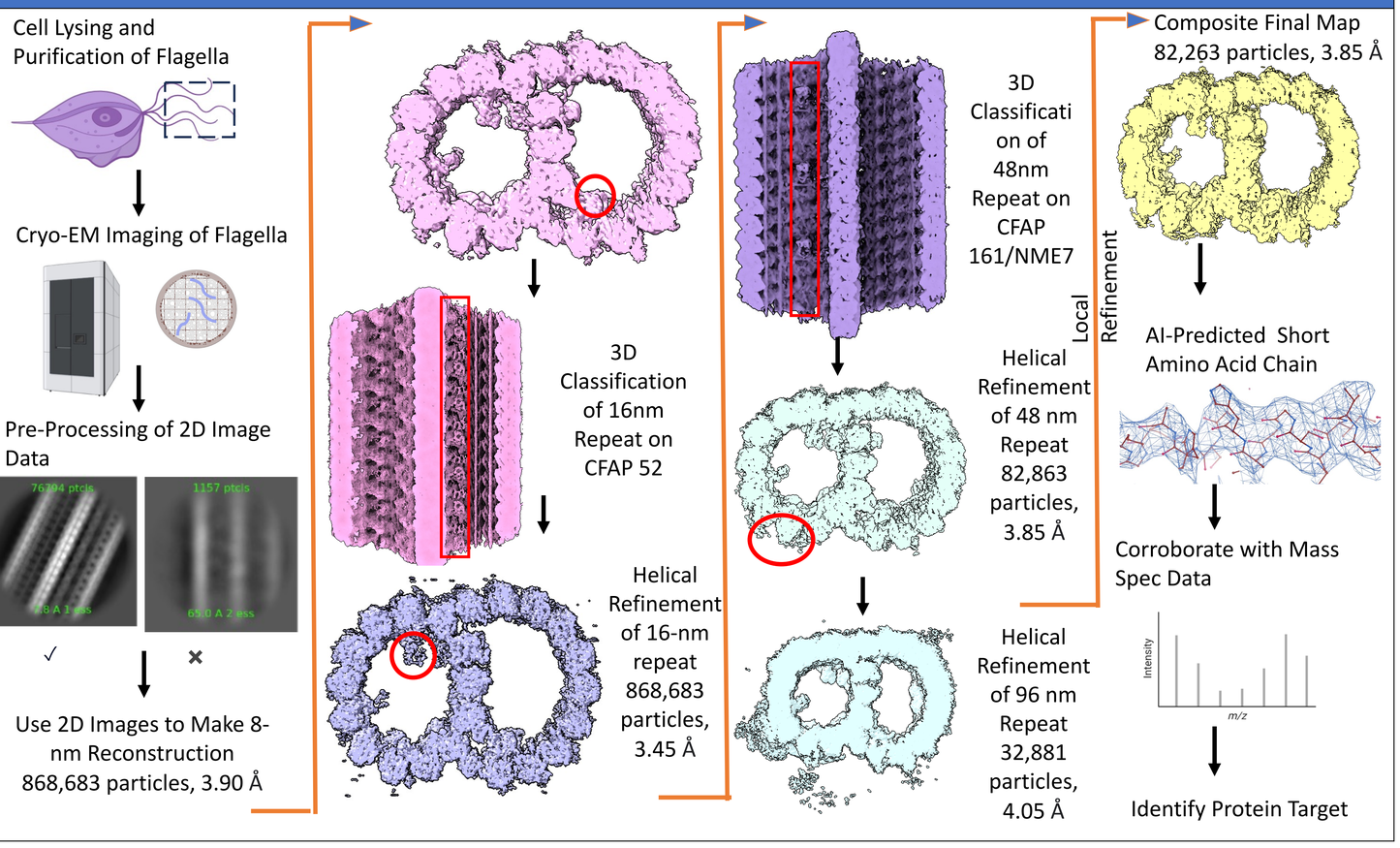
Trichomonas vaginalis (*Tv*) is a parasitic protist and causative agent of Trichomoniasis, the most common non-viral sexually transmitted infection (STI), implicated in perinatal complications and increased rates of HIV transmission. *Tv* uses its four anterior flagella, composed of 9 outer microtubule doublets (OMD) forming a ring around a central pair of microtubules, to facilitate parasite motility and host cell adherence. The absence of a high-resolution OMD structure hampers our understanding of Tv flagella and its role in drug development. To tackle this challenge, we utilized cryo-electron microscopy (cryo-EM) to image the OMDs of *Tv* flagella. Here, we performed single particle analysis to resolve a structure of the OMD to 3.8 Å resolution and identified several uncharacterized internal and external proteins. We integrated AlphaFold's AI-based protein structure predictions with mass spectrometry data to create an atomic model of the previously unidentified protein TVAG_374810. This protein exhibits homology with the CFAP91 family, implying its potential involvement in molecular ruler interactions and precise docking of radial spoke proteins, flagellar beating frequency. Altogether, this work elucidates how Tv-specific protein densities provide stability and architecture in the OMD and offers structural basis for *flagellar* movement in trichomonads.



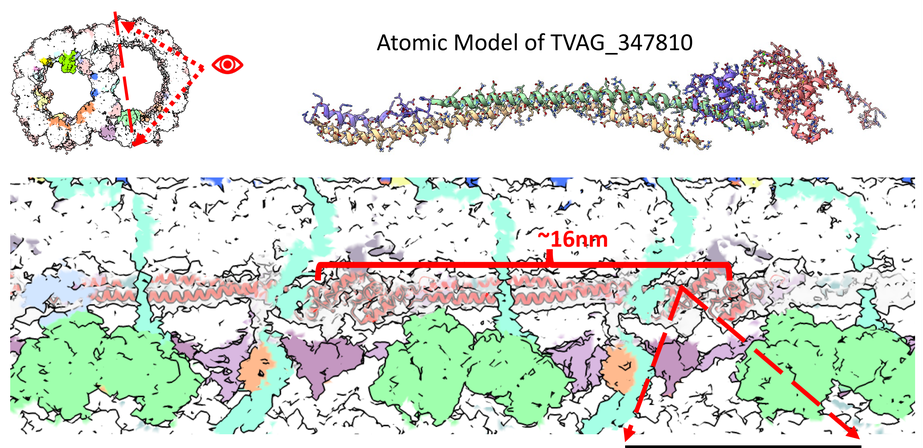
Asymmetric Reconstruction of *Tv* Outer Microtubule Doublet



Single Particle Analysis Generates High-Resolution Outer Microtubule Doublet Reconstruction

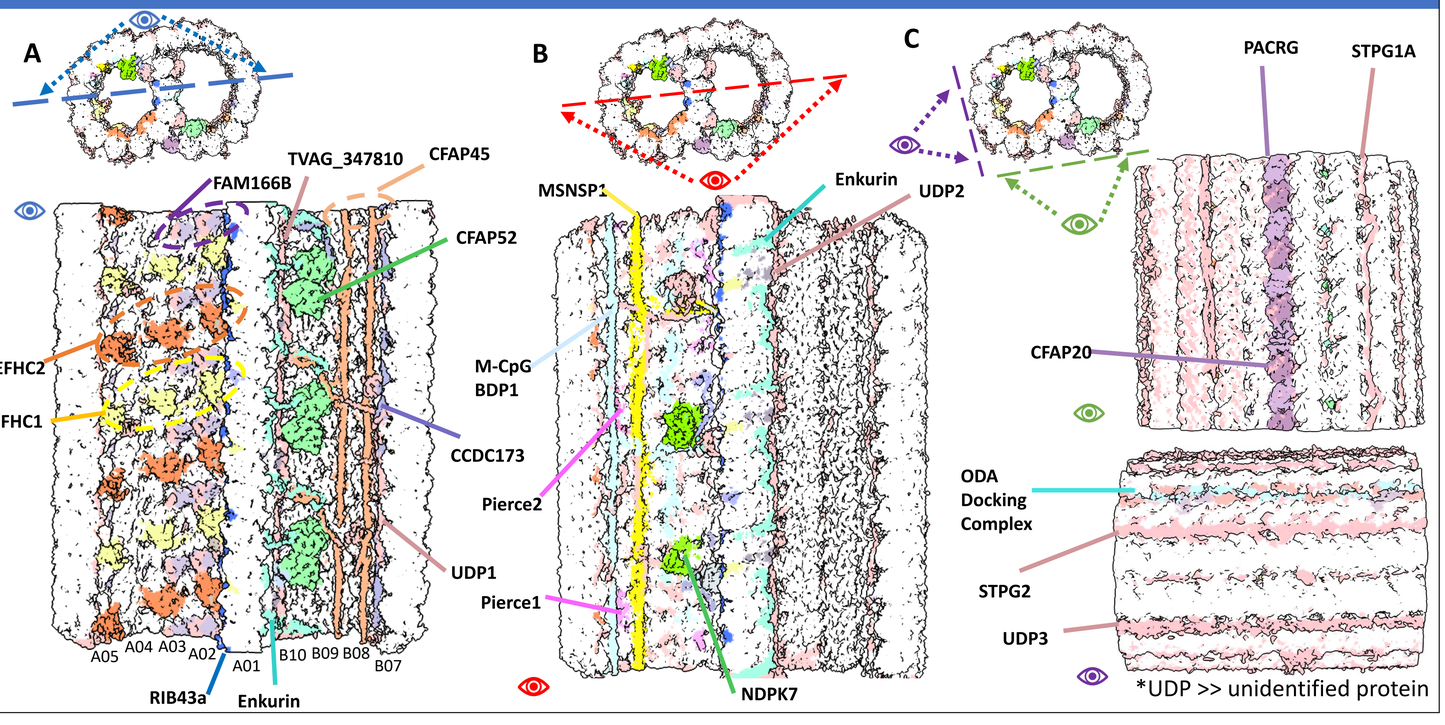


Novel Protein TVAG_347810 Identified in *Tv* Outer Microtubule Doublet



Length of Protein	377 aa
Molecular Weight	43.704 kDa
Isoelectric Point	10.19
Genomic Location	12712-12900 (+)

Single Particle Analysis Reveals Microtubule Associated Proteins Integral to Parasite Motility



Summary and Conclusion

- High-resolution Reconstruction of Trichomonas vaginalis OMD
- Refinement of 8nm, 16nm, 48nm, and 96 nm repeating densities
- Fitting of Homologous Conserved Densities from Bovine Respiratory Cilia, Mouse Sperm Doublet Microtubule
- Novel Characterization of TVAG_347810

Homologous Proteins in Bovine Respiratory Cilia | New Proteins in Tv Flagella

EHFC2	CFAP52	CFAP45	NDKP7	TVAG_347810
EHFC1	FAM166B	MSNP1	PACRG	UDP1
RIB43a	M-CpG Binding Domain Protein	Pierce1	CFAP20	UDP2
Enkurin	CCDC173	Pierce2	ODA Docking Complex	UDP3

References and Acknowledgements

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