

Engineering SPEF1 Fusion Proteins for Improved Microtubule Seam Alignment for Cryo-Electron Microscopy

Helena Goodey-Parfitt¹, Tina Alagha¹, Hayley Rose Blythe¹, Thibault Legal¹, Khanh Huy Bui¹

¹Department of Anatomy and Cell Biology, Faculty of Medicine and Health Sciences, McGill University, Montreal, QC, Canada

Keywords: Computational protein design; Cryo-electron microscopy; Fusion proteins; Microtubule seam; Microtubules; Sperm flagellar protein 1 (SPEF1)

Corresponding author email: helena.goodey-parfitt@mail.mcgill.ca

Published: 05/05/2026

<https://doi.org/10.26443/msurj.v21i2.419>

Microtubules are dynamic polymers of α -tubulin heterodimers that support cell structure and contribute to processes including cell division, intracellular transport, and cilia stability. Their functions are modulated by microtubule-associated proteins (MAPs), which regulate microtubule assembly, stability, organization, and interactions with other cellular components. Accurate identification of the microtubule seam, where heterotypic α - α interactions replace homotypic α - β and β - β contacts, is essential for cryo-electron microscopy (cryo-EM) reconstruction. Current seam-alignment strategies employ kinesin motor domains that generate alignment contrast but mask lattice features and limit compatibility with many MAPs. The Bui lab identified Sperm Flagellar Protein 1 (SPEF1) as a seam-binding alternative, though its small size limits reliable visualization. We hypothesised that engineering larger SPEF1 fusion proteins would improve seam contrast and alignment while preserving microtubule binding. Fusion constructs were designed using RoseTTAFold Diffusion to append structured domains to the SPEF1 microtubule-binding region while preserving predicted folding and function. Constructs were cloned, sequence-verified, and expressed in *Escherichia coli*, with several designs demonstrating strong expression and yielding soluble protein after purification. Using a microtubule co-sedimentation assay, the engineered construct SPEF1 (1-120)-300AA demonstrated effective binding to polymerized microtubules, supporting its suitability for further microtubule seam-targeting studies, although cryo-EM validation remains ongoing. These results position engineered SPEF1 fusion proteins as promising seam-alignment tools that provide a broadly compatible and experimentally validated strategy for advancing cryo-EM studies of microtubule architecture and enabling direct evaluation of seam localization without masking lattice features or disrupting lattice-binding protein interactions, thereby overcoming limitations imposed by existing seam-alignment strategies.

© The Authors. This abstract is published under a CC-BY license:

<https://creativecommons.org/licenses/by/4.0/>