Preliminary Results

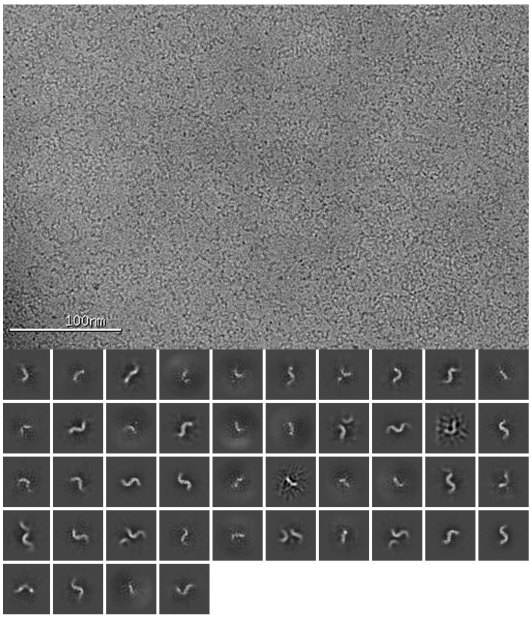

Fig. 1. 2D classes of the minimal Nup358/KLC2 complex. Top: representative cryo-electron micrograph, collected by the PI with the Krios6 cryo-TEM at NYSBC. Bottom: representative 2D classes of 1,355,298 particles from 4188 micrographs.

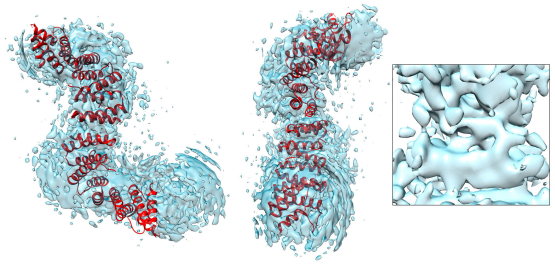

Fig. 2. Cryo-EM map of a minimal KLC2/Nup358 complex which the PI has determined at the NCCAT center; α-helices are visible. The published X-ray structure of KLC2 fused to a short LEWD peptide is docked into the map.

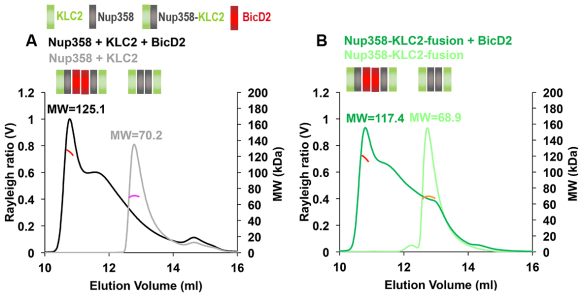

Fig. 3. BicD2-CTD, Nup358-min and KLC2 form a ternary complex with 2:2:2 stoichiometry. Proteins were analyzed by size exclusion chromatography coupled to multi-angle light scattering to determine molar masses (MW).

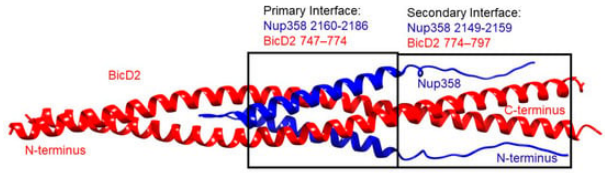

Fig. 4. Predicted structure of the minimal Nup358/BicD2 complex. This structural model from Alphafold2 was confirmed by binding assays of mutants, NMR spectroscopy, SAXS and CD spectroscopy. We recently published these results (Gibson JM, *et al.* 2022, Elife 11. doi: 10.7554/eLife.74714. Gibson JM, *et al.,* 2023. Biomolecules 13. doi: 10.3390/biom13101445).

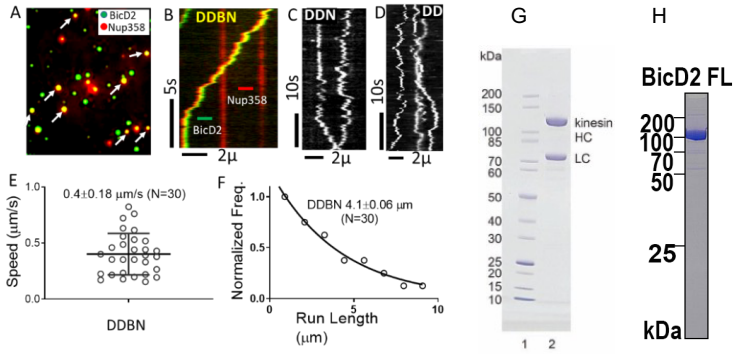

Fig. 5. The dynein/dynactin/BicD2/Nup358 complex (DDBN) moves processively on MT. Top panel: DDBN complex. **(A)** Yellow dots show that Nup358 (red) binds to BicD2 (green). **(B)** Kymograph of DDBN. **(C,D)** Both dynein/dynactin/Nup358 (DDN) and dynein/dynactin (DD) complexes diffuse on MT. **(E,F)** Speed and run length of the active DDBN complex. (**G**) SDS-PAGE of *Ms* kinesin-1 with heavy chains (HC) and light chains (LC). **(H)** SDS-PAGE of human full-length BicD2.