SPECIFIC AIMS

Nuclear positioning sustains fundamental processes in brain and muscle development. Mutations in associated proteins cause neuromuscular diseases including spinal muscular atrophy, the most common genetic cause of death in infants. The nuclear pore protein Nup358 recruits kinesin-1 and the dynein adaptor Bicaudal D2 (BicD2), which in turn recruits the dynein machinery to position the nucleus. This pathway is active in G2 phase, and it is essential for the differentiation of radial glial progenitor cells to most neurons and glia cells of the neocortex. Dynein also facilitates a vast number of cellular transport events that are critical for chromosome segregation, signal transmission at synapses, brain and muscle development. Adapters such as BicD2 are required to activate dynein for processive motility. However, in the absence of cargo, BicD2 is autoinhibited and forms a looped conformation, in which the dynein binding site is masked by the cargo binding site. Binding of cargo activates BicD2 for dynein recruitment, but the molecular mechanism of activation remains elusive. Therefore, it is important to understand: 1) how dynein adaptors such as BicD2 interact with cargo adapters, 2) how interactions between BicD2 and cargo activate dynein motility, and 3) how the opposing motor kinesin-1 modulates overall motility. To answer these questions, we plan to determine structures of autoinhibited full-length BicD2, an activated BicD2/cargo complex, and a BicD2/Nup358/kinesin-1 complex by single-particle cryo-electron microscopy (EM). Data were generated in collaboration with Kathleen Trybus, University of Vermont.

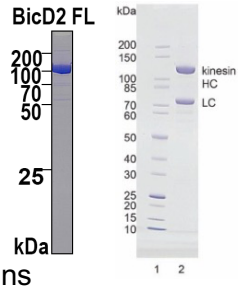

Fig. 1. SDS-PAGE of full-length *Hs* BicD2 and *Hs* kinesin-1 (HC: heavy chain; LC light chain).

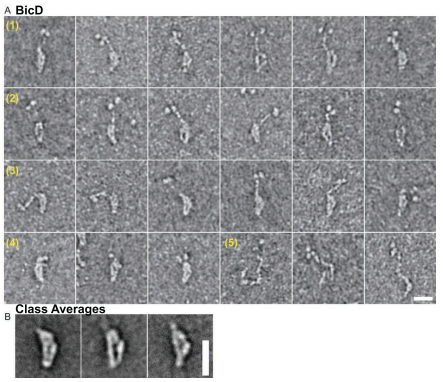

Fig. 2 (A) Negative stain EM images of *Dm* BicD. (B) 2D class averages (from Sladewski *et al.*, eLife 2018;7:e36306).

Aim (1) We plan to determine cryoEM structures of full-length autoinhibited human BicD2 and the activated F743I variant which is capable of activating dynein for processive transport in a cargo-independent manner (Cui *et al.* 2020, Traffic 21: 463) to provide insights into activation. In preparation of this study, we have purified human full-length BicD2 and the *Drosophila* homolog BicD (**Fig. 1**), which both dimerize (MW=214 kDa). Negative stain EM 2D class averages of *Dm* BicD suggest suitability for cryoEM (**Fig. 2**).

Aim (2) We plan to determine a structure of a Nup358/BicD2 complex. BicD2 recognizes cargo adapters such as Nup358, and links them to dynein. We have purified and reconstituted a complex of full-length BicD2 and a minimal domain of Nup358 (Nup358-min), which is capable of activating dynein/dynactin/BicD2 motors for processive motility (**Fig. 3**). The structure will test the hypothesis that cargo-binding opens the looped structure of BicD2 and provide insights into cargo recognition.

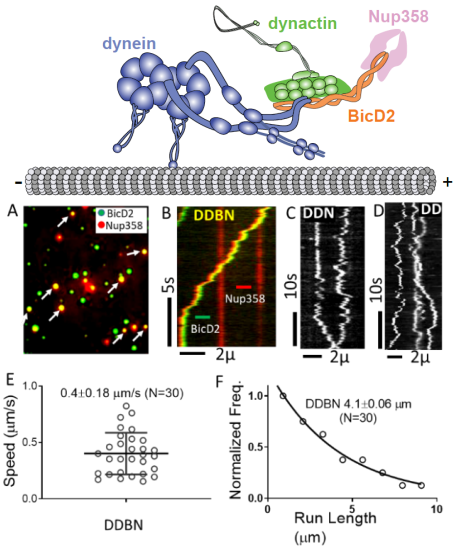

Fig. 3. Dynein/dynactin/BicD2/ Nup358 complex (DDBN) moves processively on microtubules in single molecule processivity assays. (A) Yellow dots with arrows show that Nup358 (red) binds to BicD2 (green). (B) Kymograph of DDBN (C,D) Both dynein/dynactin/Nup358 (DDN) and dynein/dynactin (DD) complexes diffuse on MT. (E,F) Speed and run length of the active DDBN complex.

Aim (3) We plan to determine a structure of a Nup358/BicD2/kinesin-1 complex. Our initial data establish that the minimal domains of BicD2 and kinesin-1 bind at separate, but closely adjacent regions on Nup358 and form a 2:2:2 complex (Cui *et al.* 2019, Biochemistry 58: 5085). Thus, we hypothesize that both dynein and kinesin-1 motors interact with Nup358 simultaneously and are coordinated to achieve precise positioning of the nucleus in the cell. We propose that the close spatial proximity promotes direct interaction between these motors, which finetune and modulate their motility. We plan to determine the structure of a minimal Nup358/BicD2/kinesin-1 complex (108 kDa) which we have characterized by small-angle X-ray scattering (SAXS) (**Fig. 4**). We have also reconstituted a 546 kDa-complex of Nup358-min, full-length BicD2, and full-length kinesin-1, which is activated for processive motility (data not shown, for SDS-PAGE see **Fig. 1**). The structures will establish how dynein and kinesin-1 motors are coordinated by adapters to precisely position the nucleus in the cell.

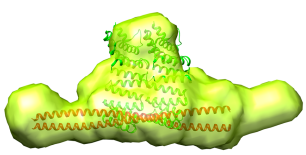

Fig 4. SAXS structure of minimal Nup358-KLC2/BicD2 complex with docked structures of BicD2 (red; PDB ID 6OFP, determined by the PI) and two KLC2s (green; PDB ID 3ZFW). NSD= 1.0 ±0.1; χ^2= 1.04; MW= 105 kDa, (MW of a 2:2 complex would be 108 kDa).