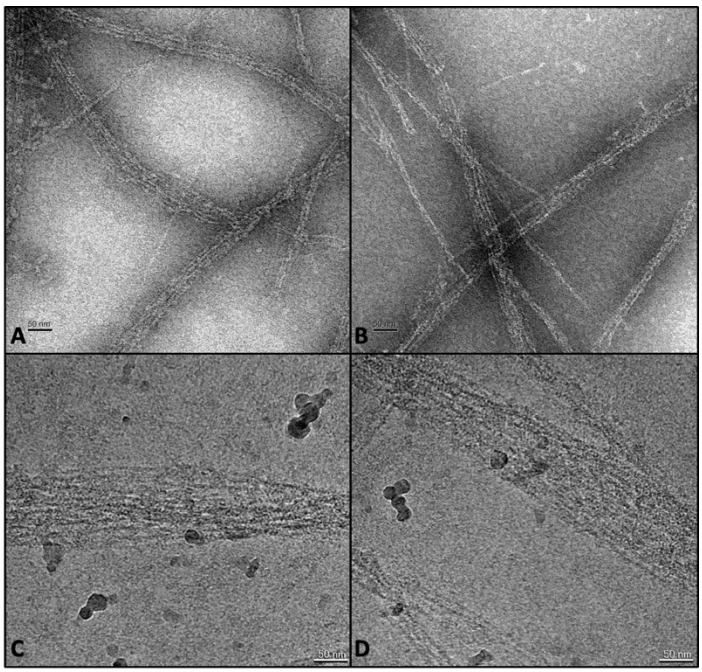

Figure 1: Micrographs of the ExoY-actin bundle. (A and B) Negative stained images showing the heterogeneity of ExoY-actin bundles. Imaged on a FEI Spirit operated at 120kV. (C and D) Images of the ExoY-actin bundle following plunge freezing using a FEI Vitrobot. Images captured using a FEI Talos operated at 200kV equipped with a Falcon II direct detector. Scale bars = 50 nm in each image.

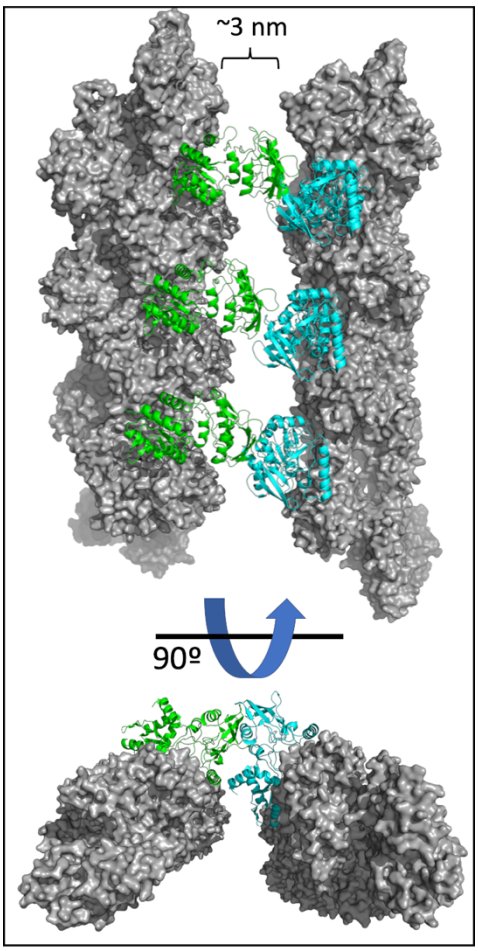

Figure 2: Model of the ExoY-actin bundle. By necessity, actin bundling requires two actin binding sites, yet only one region of ExoY is known to mediate actin binding. Therefore, we utilized protein interface surface analysis (PISA) to model a potential ExoY dimer (green and blue subunits) based upon the crystal structure of ExoY (PDB:5XNW). Previous genetic and biochemical data suggest a likely ExoY binding region on F-actin (shown as gray surface). We combined limited mutational data with charge complementarity analysis to dock the modeled ExoY dimer to two actin filaments in such a way so that the filaments run more or less parallel, as observed in the actin bundles in figure 1. This model predicts a distance of roughly 3 nm between the actin filaments. The interfilament distance is one of key aspects of this model we wish to investigate. Interfilament distances are known to vary wildly, and correlate strongly to the biological function of the actin bundle. Compact bundles with short interfilament distances often play a role in dynamic processes requiring transference of force, such as cell motility, while looser bundles with large interfilament distances are typically involved in maintaining the global cellular architecture.