Flightin (FLN) is a myosin binding protein with an essential role in determining the proper length and stiffness of thick filaments in Drosophila flight muscle. FLN is present in all species of hexapods (insects) and crustaceans who share a highly conserved, novel ~52 aa domain designated “WYR”. In addition, FLN consists of a poorly conserved N-terminal domain (~65 aa) and a C-terminal domain with intermediate conservation (~42 aa). It has been found that deletions of the C-terminal and N-terminal domains in transgenic models are not necessary for FLN accumulation in the thick filament. The purpose of this study is to identify the FLN sequences necessary for myosin binding and to understand how the FLN-myosin interaction confers stiffness to the thick filament. We hypothesize that the WYR domain in invertebrate myosin binding proteins engages in a functionally homologous role to the IgC2 domain in vertebrate myosin binding protein C MyBP-C). Both full FLN and the IgC2 CX domain of MyBP-C have been shown to bind to a common region of the myosin light meromyosin (LMM) domain. Here, we have generated a series of fusion constructs expressing different combinations of FLN domains to test their LMM binding using a co-sedimentation assay. Studies are currently underway to establish the parameters for optimal protein solubility and measuring the interaction between proteins using this method.