MS08-05 | Structure Of A-Actinin-2/Fatz-1 Fuzzy Complex And Implications In Z-Disk Formation Via Phase Separation

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α-Actinin-2 plays a pivotal role in Z-disk assembly and stability as it crosslinks actin filaments from adjacent sarcomeres and acts as a binding platform for a number of Z-disk proteins. Among them is FATZ-1, a 30-kDa protein that appears when Z-bodies, the precursors of Z-disks, are formed. Accordingly, FATZ-1 is believed to have a central role at initial stages of myofibrillogenesis by serving as focal point for interactions with Z-disk proteins, but its structure and function remain unknown.

Here, we used bioinformatics, CD and SAXS to show that FATZ-1 is intrinsically disordered after generating a soluble construct (D91 FATZ-1). We next studied the affinity and binding stoichiometry of a-actinin-2/FATZ-1 complex by ITC and SEC-MALS, and mapped down a shortest construct (mini FATZ-1) using XL/MS, NMR and LP/MS, as the disordered nature of this protein was a challenge for our crystallographic studies. We then managed to solve the structure of α-actinin-2 rod/mini FATZ-1 complex at 2.7Å and α-actinin-2 half dimer/D91 FATZ-1 complex at 3.2Å using MR and Se-Met variants of FATZ-1. In addition, we modelled the flexible regions of D91 FATZ-1 in complex with α-actinin-2 by SAXS to have a complete molecular model of the complex. Finally, we studied FATZ-1 capacity to phase separate and form liquid droplets in the presence of α-actinin-2. Together, our results provide a complete and detailed structural model of the α-actinin-2/ FATZ-1 fuzzy complex as well as a plausible model on how FATZ might attract Z-disk proteins and form membrane-less compartmentalization at initial stages of myofibrillogenesis.