MS08-P02 | Structure Of The A-Actinin Actin-Binding Domain/F-Actin Complex

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α-Actinin plays a crucial role in cytoskeleton organization by crosslinking actin filaments. All four human α-actinin isoforms consist of an N-terminal actin binding domain (ABD; 252-271 residues) comprising two calponin homology domains (CH1 and CH2) connected each other via a flexible linker. Accordingly, mutations on ABD that increase or decrease binding to F-actin lead to disease. Here, we took advantage of a high-affinity, disease-associated α-actinin-4 mutant (K255E) to obtain a 4.2 Å cryo-EM structure of the ABD/F-actin complex. We confirmed previously reported actin biding sites present in CH1, but could not observe CH2 most likely due to orientational flexibility. We could not observe the N-terminal part of the ABD either which is predicted to be disordered in all α-actinin isoforms based on bioinformatics studies. To examine the role of this N-terminal part, which ranges from 10 residues in α-actinin-1 to 28 residues in α-actinin-4, we performed actin cosedimentation assays with different N-terminally truncated constructs of all α-actinin isoforms. We further investigated ABD/F-actin interaction by XL/MS and NMR which proved that not only CH1 but also CH2 is involved in binding to F-actin. Taken together, our results provide a complete molecular model of the α-actinin ABD/F-actin complex and highlight the importance of the N-terminal part in direct interaction with F-actin.