

MS08-04 | STRUCTURAL BASIS OF α -ACTININ-2/TITIN INTERACTION IN THE Z-DISK

Lopez Arolas, Joan (Max F. Perutz Laboratories, University Vienna, Vienna, AUT); Sponga, Antonio (Max F. Perutz Laboratories, University Vienna, Vienna, AUT); Smith, Luke (King's College London, London, GBR); Gautel, Mathias (King's College London, London, GBR); Djinovic-Carugo, Kristina (Max F. Perutz Laboratories, University Vienna, Vienna, AUT)

α -Actinin-2 plays a central role in Z-disk assembly and stability as it crosslinks actin filament thanks to its unique antiparallel dimeric architecture. The organization and spacing between each α -actinin-2 dimer is regulated via two specific interactions with titin, the giant blueprint protein of the sarcomere: (i) α -actinin-2 EF3-4 interact with specific titin Z-repeats in a PIP2-regulated manner and (ii) α -actinin-2 rod domain interacts with Zq titin. The first interaction has been studied in detail at all levels, but the molecular basis behind the second interaction as well as on how the two binding events translate into Z-disk assembly remain unknown.

Here, CD and SAXS analyses of Zq and Zr7-Zq titin constructs revealed that they are intrinsically disordered. Subsequent ITC experiments showed the binding affinity of rod/Zq and EF3-4/Zr7 plus rod/Zq binding events. We next managed to crystallize rod/Zq and solve its structure to 2.8 Å resolution using MR, data from XL/MS and PMF MS of the crystals, as well as Se-Met labeled variants of Zq mutants. We further validated rod/Zq interacting regions by generating different mutants whose effect is currently being analyzed in muscle cells. In addition, we modelled the flexible regions of Zr7-Zq in complex with α -actinin-2 using SAXS to have a complete molecular model of the complex. Finally, we designed a collection of MBP-fused titin constructs and are currently analyzing their complexes with α -actinin-2 using SEC-MALS and NS EM. Altogether, our results point to an important role for Zq in α -actinin-2/titin interaction which might regulate proper Z-disk assembly.