MX-241: "Molecular basis of muscle elasticity"

Under this proposal we have completed:

A) Structure elucidation of A168-A170, a 3-domain fragment from the M-line of titin
B) Structure elucidation of I65-I70, a 6-domain fragment from the elastic I-band of titin
C) Structure elucidation of I67-I69, a 3-domain fragment from the elastic I-band of titin
D) Structure elucidation of I67–I69^{E93A}, a mechanical mutant of I67-I69

PDB accession codes: 2NZI, 3B43, 2RIK, and 2RJM

Manuscripts:

1**. Mrosek M, Labeit D, Witt S, Heerklotz H, von Castelmur E, Labeit S, Mayans O. (2007) "Molecular determinants for the recruitment of the ubiquitin-ligase MuRF-1 onto M-line titin." FASEB J. 21(7):1383-92.**

Abstract: Titin forms an intrasarcomeric filament system in vertebrate striated muscle, which has elastic and signaling properties and is thereby central to mechanotransduction. Near its C-terminus and directly preceding a kinase domain, titin contains a conserved pattern of Ig and FnIII modules (Ig(A168)-Ig(A169)-FnIII(A170), hereby A168-A170) that recruits the E3 ubiquitin-ligase MuRF-1 to the filament. This interaction is thought to regulate myofibril turnover and the trophic state of muscle. We have elucidated the crystal structure of A168-A170, characterized MuRF-1 variants by circular dichroism (CD) and SEC-MALS, and studied the interaction of both components by isothermal calorimetry, SPOTS blots, and pull-down assays. This has led to the identification of the molecular determinants of the binding. A168-A170 shows an extended, rigid architecture, which is characterized by a shallow surface groove that spans its full length and a distinct loop protrusion in its middle point. In MuRF-1, a C-terminal helical domain is sufficient to bind A168-A170 with high affinity. This helical region predictably docks into the surface groove of A168-A170. Furthermore, pull-down assays demonstrate that the loop protrusion in A168-A170 is a key mediator of MuRF-1 recognition. Our findings indicate that this region of titin could serve as a target to attempt therapeutic inhibition of MuRF-1-mediated muscle turnover, where binding of small molecules to its distinctive structural features could block MuRF-1 access.

2. von Castelmur E, Marino M, Svergun DI, Kreplak L, Ucurum-Fotiadis Z, Konarev PV, Urzhumtsev A, Labeit D, Labeit S, Mayans O. (2008) "A regular pattern of Ig super-motifs defines segmental flexibility as the elastic mechanism of the titin chain." Proc Natl Acad Sci U S A. 105(4):1186-91.

Abstract: Myofibril elasticity, critical to muscle function, is dictated by the intrasarcomeric filament titin, which acts as a molecular spring. To date, the molecular

events underlying the mechanics of the folded titin chain remain largely unknown. We have elucidated the crystal structure of the 6-Ig fragment I65-I70 from the elastic I-band fraction of titin and validated its conformation in solution using small angle x-ray scattering. The long-range properties of the chain have been visualized by electron microscopy on a 19-Ig fragment and modeled for the full skeletal tandem. Results show that conserved Ig-Ig transition motifs generate high-order in the structure of the filament, where conformationally stiff segments interspersed with pliant hinges form a regular pattern of dynamic super-motifs leading to segmental flexibility in the chain. Pliant hinges support molecular shape rearrangements that dominate chain behavior at moderate stretch, whereas stiffer segments predictably oppose high stretch forces upon full chain extension. There, librational entropy can be expected to act as an energy barrier to prevent Ig unfolding while, instead, triggering the unraveling of flanking springs formed by proline, glutamate, valine, and lysine (PEVK) sequences. We propose a mechanistic model based on freely jointed rigid segments that rationalizes the response to stretch of titin Ig-tandems according to molecular features.