NMR studies of the large super-repeats in titin

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Introduction

Titin is the largest protein yet found (chain weight ~3 MDa) and the third most abundant protein of muscle, after myosin and actin. Titin molecules are ~1 μm long and span between the Z- and M-lines in muscle sarcomeres. Most of the molecule consists of two classes of concatenated ~100 residue domains similar to I-set immunoglobulins and type III fibronectins. In the A-band titin is integral with the thick filament, where it is proposed to regulate exact assembly to the 294 myosin molecules present. In this region the Ig and Fn domains are arranged in 11 domain patterns, Ig-Fn-Fn-Fn-Ig-Fn-Fn-Fn-Ig-Fn-Fn, called large super-repeats, which are themselves repeated 11 times. Each super-repeat spans the 43 nm helical repeat of the thick filament. The entire 121 domain region is nearly half the ~300 domains making up titin. The size and stability of these Ig and Fn domains make them very suitable for structure studies by NMR, while it is the method of choice to gain insight in interdomain flexibility and the behavior of interdomain linker sequences.

Results

We have expressed in *E. coli* seven 2 and 3 domain overlapping constructs spanning one large super-repeat and we are studying the structure and flexibility of these. Figure 1 shows the ^{15}N–^{1}H HSQC-TROSY spectrum of the A67-A68 tandem illustrating the quality of the

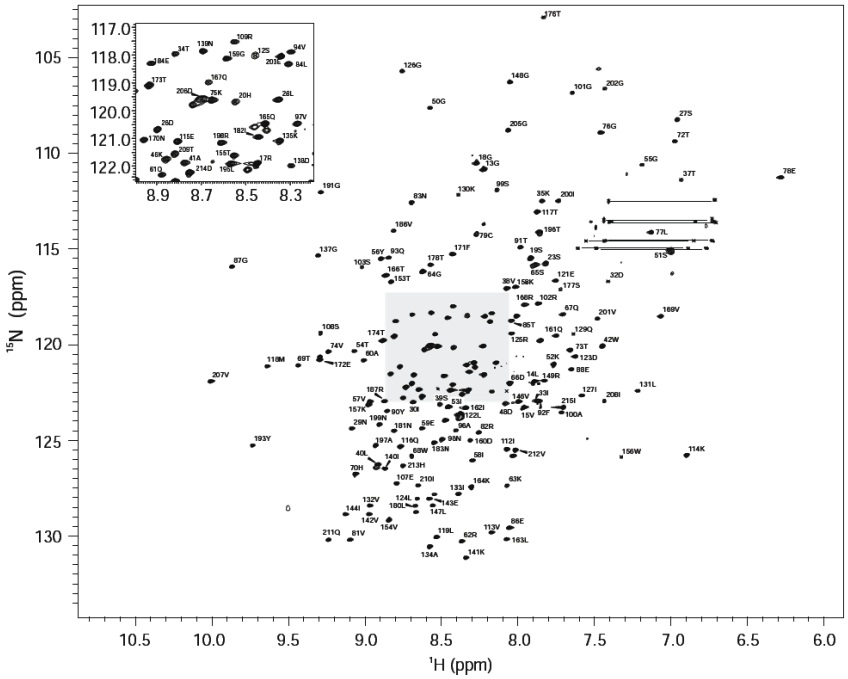

Figure 1: two-dimensional H–N HSQC-TROSY spectrum of the A67-A68 tandem at 750-MHz H frequency. Data were acquired at 293K using a uniformly ^{15}N, ^{13}C, ^{2}D labelled protein sample (~1 mM).

data and breadth of assignment. The spectrum show strong signals with uniform intensities, indicative of a well defined three-dimensional structure and appears to tumble as a single unit. The completeness of the assignments of A67-A68 tandem are 93.4% (170/182) for backbone ^{1}HN and ^{15}N, 94.9% (185/195) for $^{13}C\alpha$, 92.3% (167/ 181) for $^{13}C\beta$, and 86.7% (169/195) for $^{13}C0$ resonances. Most of the unassigned resonances were in loop regions. The chemical shift and TALOS data indicate the presence of fourteen β-strand regions from the

two domains. Determination of a high resolution NMR structure and a detailed description of the extent of inter-domain flexibility are in progress.

Publications

Czajlik, A., Thompson, G., Khan, G., Kalverda, A., Homans, S. & Trinick, J. (2012) H-1, N-15 and C-13 backbone chemical shift assignment of the titin A67-A68 domain tandem. *Biomol. NMR Assign.* **6:** 39-41.

Tskhovrebova, L. & Trinick, J. (2012) Making muscle elastic: the structural basis of myomesin stretching. *PLoS Biol.* **10:** e1001264-e1001264.

Tskhovrebova, L. & Trinick, J. (2012) Molecular rulers? *Curr. Biol.* **22:** R317-R318.

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