Structure of a Titin/Obscurin-like 1 Complex: Solved using an Agilent Xcalibur Nova X-ray Diffractometer

Providing structural insights into muscle M-band assembly and mechanics

Application Note

X-ray Crystallography

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Data kindly provided by Dr Roberto Steiner

For more details see: Pernigo, *et al*. (2010) Proc *Natl Acad Sci USA*, **107**: 2908-2913.

Introduction

To understand at the atomic level the sarcomeric M-band interaction between titin and obscurin-like 1 (Obsl1), the minimal interacting complex was in *vitro* reconstituted and its X-ray crystal structure solved in two different crystal forms (Form I, trigonal and Form II, triclinic— PDB codes 2wp3 and 2wwm, respectively). Titin M10 and the N-terminal immunoglobulin (Ig) module of human Obsl1 (OL1) form a heterodimer in solution and in the crystal. No major structural differences are seen in the two different crystal forms, suggesting that the complex observed crystallographically faithfully recapitulates that in solution. Both M10 and OL1 protomers belong to the intermediate set (I-set) of the Ig family and share the typical barrel-like architecture in which a total of nine strands are arranged into two distinct β-sheets folded into a β-sandwich.

Figure 1. The Agilent Nova Cu micro-focus X-ray source

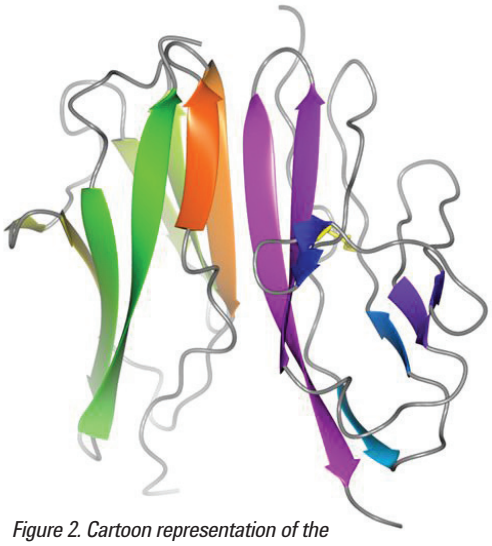

Figure 2. Cartoon representation of the M10-OL1 heterodimer structure

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By using an integrated structural, biophysical, and cell biological approach the authors illustrated how the titin M10 domain interacts with the N-terminus of obsl1, suggesting a molecular explanation for the differential affinity of obscurin and Obsl1 to titin M10. They also provided a basis for a mechanistic understanding of the titin mutations linked to two hereditary muscle diseases: limb girdle muscular dystrophy 2J and tibial muscular dystrophy.

Experimental details

Crystals of the M10-OL1 complex were grown by the hanging drop method at 18 °C either in the presence of 0.10M Bis-Tris pH 5.5, 2.0M ammonium sulphate (Form I, trigonal) or in the presence of 0.10M MIB buffer pH 5.0, 25% (w/v) PEG 1500 (Form II, triclinic).

For data collection crystals were cryoprotected by soaking them in their respective reservoir solutions supplemented with 20% glycerol. An initial dataset on a M10-OL1 complex trigonal form crystal was collected in-house on an Agilent (formerly Oxford Diffraction) Xcalibur Nova system (Fig 1) at the 2.3 Å resolution and processed using the Agilent CrysAlis^{Pro} software package. The data collection statistics are shown in Table 1.

This dataset was used to solve the structure of the M10-OL1 complex in the trigonal $P3_1$ space group by the molecular replacement method using the program MOLREP and employing the structure of telokin (PDB code 1FHG) as search model. A clear solution was obtained with one M10-OL1 complex in the asymmetric unit. High-resolution data were later collected at the Diamond Light Source for final rounds of structure refinement carried out using the program REFMAC5. After restrained refinement, the best M10-OL1 model (Fig 2) refined using the synchrotron data is characterized by an R_{free} value of 21.7% and excellent quality indicators.

	Overall	Inner Shell	Outer Shell
Low resolution limit [Å]	20.11	20.11	2.42
High resolution limit [Å]	2.30	7.27	2.30
Rmerge	0.041	0.024	0.162
Total number of observations	20259	594	2936
Total number unique	8002	241	1177
Mean((I)/sd(I))	23.1	69.2	5.0
Completeness [%]	99.7	94	99.6
Multiplicity	2.5	2.5	2.5

Table 1. X-ray data collection statistics for data collected on an Agilent Xcalibur Nova system

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