Carving a Beanstalk: the Structure of ΔS2 from Human Myosin II

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S2 is the flexible coiled coil that connects light meromyosin to the N-terminal motor domain of myosin II. S2 interacts with other proteins of thick filament and can lead to fatal familial hypertrophy (FFH) when mutated. We have determined the structure of a 126-residue N-terminal fragment of S2 in two different crystal forms. The WT protein diffracted to 2.7 Å resolution in a $C222_1$ cell of a=40, b=46, c=373 Å. Cryo-protection was difficult and data could only be reduced in XDS. Phases were derived from 2-λ MAD data collected from a mercury derivative. Only SHELXD with SHARP generated interpretable electron density maps. The protein is a parallel dimeric coiled coil of 187 Å lying stretched out along the c-axis.

The FFH-associated E924K-mutant crystallised in P1 with a=40, b=42, c=98 Å; α=91, β=93, c=107°. Molecular replacement was not successful and crystals were highly radiation sensitive, giving non-traceable electron density maps when anomalous phasing from SeMet-labelled or heavy-atom-soaked crystals was employed. It was, however, possible to locate 4 mercury atoms from anomalous data. These co-ordinates together with the position of cysteine residues in the WT structure were used in a semi-brute-force approach to derive the relative orientation of two coiled coils in the asymmetric unit. The model was refined to 2.5 Å with R=27.3 and R_{free}=34.9 %. The two extended coiled coils run anti-parallel with neighbouring molecules lying head-to-tail such that they form quasi-endless filaments.

Keywords: coiled coil proteins, MAD, brute force