MX-241
Molecular basis of muscle elasticity

Under this proposal we have carried out:

1. Structure elucidation of X114: a 3-domain fragment from titin responsible for force response. **Status: completed**

The crystallization yield of this construct was extremely low and crystals of sufficient quality for X-ray data collection were only obtained in one single drop. Two crystals were available in total in the habit of long rods of up to 1mm length. Native data to 2.85 Å resolution were collected at ID-29 in the 3.Nov.2004 visit. Phasing was initially attempted by molecular replacement but this approach did not yield interpretable electron density maps. Next, phasing was attempted by double Se-Met/Se-Cys labeling, to compensate for the low occurrence of these amino acids in the titin fragment. However, successful phasing by this method was precluded by low Se incorporation, presumably at the Cys positions. Phasing was achieved via 3l-MAD on a Ta_6Br_{12} derivative obtained by soaking of a crystal fragment excised from the same crystal employed in native data collection. The MAD measurements took place in ID-23 during the 9.Dec.2004 visit. This used peak and inflexion wavelengths at the L-III edge of Ta together with a very distant high-energy remote outside the ragged L-edge area. Derivative data extended up to 3.5 Å resolution. It could be then established that the crystals belong to space group $P3_121$ with two molecules per asymmetric unit, where an imperfect two-fold NCS axis is aligned parallel to the three-fold crystallographic axis and contained within the *ac* plane. The crystals have relatively large unit cell dimensions of a=b=126.1 Å, c=134.3 Å and contain about 75-80% solvent content, explaining why data of workable resolution could only be obtained from the largest crystal grown. The model is currently in its final stages of refinement.

2. Structure elucidation of a 3-domain fragment from the elastic I-band of the titin filament. This fragment is directly involved in passive force mechanics. **Status: completed**

This is the first significant poly-domain from titin for which structural data are available. Furthermore, it is the first elastic fragment to be analyzed at atomic detail. Native data to 1.6 Å resolution were collected in ID-29 during the visit in 3.Nov.2004. Structure refinement is completed.

Manuscript in preparation:
Marino M, von Castelmur E, Kreplak L, Chami M, Svergun D, Labeit S, Mayans O. "Architectural basis of spring mechanisms in the giant elastomer titin responsible for muscle elasticity"

3. Native and derivative data were collected for 4 and 6 domain fragments up to about 3.3 Å resolution during the 3.Nov.2004 and 9.Dec.2004 visit. Surprisingly, structure elucidation has not yet been completed for these fragments. Several possibilities are now being explored, such as twinning of the data and several symmetry problems. **Status: on-going**