

## MS.23.4

*Acta Cryst.* (2011) A67, C65**Phasing of an unpredicted palindromic coiled-coil motif**

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Coiled-coil (CC) motifs are simple folds consisting of two to five amphipathic helices that wind around each other to form a supercoil. The sequences of the integrating helices contain repeats of seven- or eleven-residues, often biased in composition [1]. CC motifs are amongst the most ubiquitous folds found in proteins, where they have roles in mediating self- and hetero-association, folding and molecular recognition. The importance of CC folds is further enhanced by the fact that, through the design of synthetic sequences, their properties are exploited in the development of self-assembled and bioactive polymeric materials.

Despite the apparent simplicity of CC architectures, their crystallographic analysis remains a challenge. Factors contributing to this are: *i*) their filamentous nature and individualized helical twist that confers them a varying degree of long-range order and complicates molecular replacement approaches through cumulative deviations along the molecular axis of the superhelix; *ii*) the formation of idiosyncratic crystal lattices resulting from their acutely anisometric molecular shape; *iii*) the repetitive nature of their sequences that are often deficient in methionine and cysteine residues that could assist MAD phasing; and, most importantly, *iv*) the acute mutability of their self-association properties that are highly dependent on medium conditions and exact sequence content causing drastic and unpredictable changes of their overall conformation and troubling the interpretation of electron density maps. These factors often act in combination to trouble the structural elucidation of this important class of proteins.

Recently, we have elucidated the crystal structure of a core CC fragment, CC<sub>7</sub>, from a muscle-specific E3 ubiquitin ligase involved in the response of the muscle tissue to mechanical stimulation [2]. The structure reveals a novel, tetrameric, off-registry, palindromic arrangement not previously observed in this fold class. In this, two parallel dimeric CC (each resembling a pair of open scissors) intertwine into each other through their C-terminal ends to form an inverted tetramer, where the shared tetrameric fraction contains an aromatic hydrophobic core, rarely found in CC motifs. Such inverted arrangement is possible because the parallel chains that compose each of the CC dimers are offset by exactly one heptad repeat at the sequence level.

Crystals of CC<sub>7</sub> were obtained in three distinct crystal habits, all arising from closely related mother liquor condition: needles (P2<sub>1</sub>2<sub>1</sub>2<sub>1</sub>), plates (P2<sub>1</sub>) and bipyramides (apparent symmetry P4<sub>1</sub>22/P4<sub>3</sub>22 arising from merohedral twinning with twinning fraction >0.4). Diffraction data were obtained from all symmetries, with the maximal resolution collected being 2.1 Å for P2<sub>1</sub> plate crystals. Attempts at phasing using MR, MIRAS or MAD on cluster derivatives proved unproductive. Finally, the fragment-based MR approach implemented in Arcimboldo [3] was successful in overcoming phasing problems and providing an initial, workable model. In this contribution, we will offer an analysis of the factors that troubled the elucidation of this CC motif and will place this in the context of persistent difficulties with this protein fold.

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**Keywords:** coiled-coil motif, fragment-based phasing

## MS.23.5

*Acta Cryst.* (2011) A67, C65**S-SAD phasing of protein o-fucosyltransferase-1at a resolution of 2.6 Å in the monoclinic space group C2**

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Protein O-fucosylation is an essential post-translational modification, involved in embryonic development, adult tissue homeostasis and folding of proteins. Two different enzymes are responsible for this modification, Protein O-fucosyltransferase 1 and 2 (POFUT1 and POFUT2, respectively). Both enzymes have been characterized biologically and enzymatically but nothing is known at molecular level. A catalytically functional POFUT1 (356 residues) in an apo-form has been crystallized in the monoclinic space group C2. The crystals have been notoriously difficult to derivatise and up to 30 different heavy atoms were tried without any significant anomalous signal. Two data sets were taken in order to solve the structure: a highly redundant data set collected in-house on a Bruker microsource (with a Kappa goniometer and an Axiom detector) to 2.6 Å resolution; and a high resolution data set collected at a synchrotron source (collected at BM16 in the ESRF at wavelength of 0.99 Å) to 1.55 Å resolution. The determination of the structure by Sulphur – Single Anomalous Diffraction (S-SAD) phasing has proven to be challenging at two stages: the determination of the substructure and the phase extension to high resolution. The successful procedure for structure determination by S-SAD will be presented as well the application of this procedure to a few additional novel and test proteins.

**Keywords:** s-sad, pofut1, phasing

## MS.24.1

*Acta Cryst.* (2011) A67, C65-C66**Molecular tectonics: Design of enantiomerically pure tubular crystals**

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The preparation of chiral porous solids is of interest for chiral transformation. However, the design of crystalline materials displaying enantiomerically pure cavities or channels still remains a challenge. A possible strategy for the generation of such materials may be based on crystals composed of helical strands provided that the dimension of the helical space can be controlled and its handedness imposed. Several examples of helical metal-organic frameworks have been reported. Although the majority of infinite helical architectures described is composed of 1-D networks. examples of 2-D and 3-D helical networks have been also published.

Using the molecular tectonics approach [1] coordination networks [2] may be obtained by combining metallatectons, i.e. metal centres or metal complexes offering at least two free coordination sites oriented in a divergent manner with organic coordinating tectons possessing at least two divergently oriented ligating sites. For the design of helical coordination networks, one may combine ZnSiF<sub>6</sub>, forming in the presence of organic tectons an infinite pillar by bridging consecutive zinc cations by SiF<sub>6</sub><sup>2-</sup>, anions through Zn-F bonds [3].

The formation of enantiomerically pure 2-D helical coordination networks based on combinations of rigid enantiomerically pure bisonodentate tectons with ZnSiF<sub>6</sub> will be presented and discussed.