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Acknowledgement: this work was supported by the post-doctoral grant SFRH/BPD/30142/2006

Keywords: hemoprotein, enzyme, x-ray

MS93.P07

Acta Cryst. (2011) A**67,** C770

Functional studies on disulfide bond forming proteins of *Wolbachia pipientis*

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To function correctly, proteins need to be folded into their correct three-dimensional structure. For secreted proteins, a key step is the introduction of disulfide bonds between cysteine residues: disulfide bond forming (Dsb-) proteins are crucial in this step. E. coli has one of the best-characterized Dsb machineries involving two pathways, the oxidative and the isomerase pathways. While EcDsbA introduces disulfide bonds into proteins in the oxidative pathway with the help of EcDsbB, EcDsbC corrects misfolded proteins by reshuffling in the isomerase pathway [1]. Even though E. coli demonstrates a well-established model, the Dsb-pathway for other organisms including *Wolbachia pipientis* remains unclear. *Wolbachia pipientis*, a Gram-negative -proteobacterial endosymbiont, is able to infect more than 65% of all insect species [2]. Most interestingly, it has the unique ability to alter the reproduction of its host in several ways to favour its own transmission [3]. The molecular interaction between Wolbachia and its host implies that disulfide bond proteins could be involved in folding of transmitter factors. Indeed, Wolbachia's *w*Mel genome encodes three Dsb proteins, α-DsbA1 and α-DsbA2 and an integral membrane protein α-DsbB [4]. I have biochemically characterized the two soluble Dsb proteins and the membrane protein α-DsbB. α-DsbB is predicted to be similar to EcDsbB and I have shown that it uses a similar electron pathway. I have also shown that α-DsbB needs all four cysteines and exogenous ubiquinone1 to actively oxidize α-DsbA1. However it does not oxidize α-DsbA2. The binding mode is likely to be different from that of EcDsbB to EcDsbA because α-DsbA1 lacks a hydrophobic groove. I have crystallized α-DsbB using the cubic phase method. I was also able to form a stable complex which is being used in crystallization trials. The structures of α-DsbB and the α-DsbB:α-DsbA1 complex will help to shed light on their interaction mode.

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Keywords: oxidases, membrane protein, biochemical assays

MS93.P08

Acta Cryst. (2011) A**67,** C770

Structure of the C-terminal domain of the MutL homolog from *N. gonorrhoeae*.

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The mismatch repair (MMR) pathway serves to maintain the integrity of the genome by removing mispaired bases from the newly synthesized strand. In *E. coli*, MutS, MutL and MutH co-ordinate to discriminate the daughter strand through a mechanism involving lack of methylation on the new strand. This facilitates the creation of a nick by MutH in the daughter strand to initiate mismatch repair. Many bacteria and eukaryotes –including humans- do not possess a homolog of MutH. Although the exact strategy for strand discrimination in these organisms is yet to be ascertained, the required nicking endonuclease activity is resident in the C-terminal domain of MutL. This activity is dependent on the integrity of a conserved metal binding motif. Unlike their eukaryotic counterparts, MutL in bacteria like *Neisseria* exist in the form of a homodimer. Even though this homodimer would possess two active sites, it still acts a nicking endonuclease. Here, we present the crystal structure of the C-terminal domain (CTD) of the MutL homolog of *Neisseria gonorrhoeae* (NgoL) determined to a resolution of 2.4 Å. The protein crystallized in the space group P2(1) with cell dimensions of a = 49.5 Å, b = 62.1 Å, c = 92.1 Å and $\alpha=\gamma=90^{\circ}$, $\beta=104.6^{\circ}$. The structure shows that the metal binding motif exists in a helical configuration and that four of the six conserved motifs in the MutL family - including the metal binding site- localize together to form a composite active site. NgoL-CTD exists in the form of an elongated inverted homodimer stabilized by a hydrophobic interface rich in leucines. The inverted arrangement places the two composite active sites in each subunit on opposite lateral sides of the homodimer. Such an arrangement raises the possibility that one of the active sites is occluded due to interaction of NgoL with other protein factors involved in MMR. The presentation of only one active site to substrate DNA will ensure that nicking of only one strand occurs to prevent inadvertent and deleterious double stranded cleavage.

Keywords: mismatch repair, mutL, homodimer

MS93.P09

Acta Cryst. (2011) A**67,** C770-C771

Structure and mechanism of the diiron benzoyl coenzyme A epoxidase BoxB

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A recently elucidated coenzyme A (CoA) dependent aerobic benzoate metabolic pathway [1] uses an unprecedented chemical strategy to cope with the high resonance energy of aromates by forming the non-aromatic 2,3-epoxybenzoyl-CoA [2]. The crucial dearomatizing and epoxidizing reaction is carried out by BoxB and the two required electrons are delivered by BoxA, a NADPH dependent