MS5-P12 Structural analysis of HD-PTP phosphatase and complexes with ESCRTs

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HD-PTP/PTPN23 is a tumour suppressor phosphatase that sits at the crossroads of three essential processes; endocytosis, down-regulation of mitogenic receptors, and cell migration. HD-PTP is essential for down-regulation of activated EGFR by coordinating its trafficking to the lysosome for degradation. This role involves the specific recruitment of three different endosomal sorting multi-protein complexes, ESCRTs, that control the formation of the intralumenal vesicles to which EGFR is sorted. However, the molecular mechanism that regulates the exchange between ESCRTs is still unkown. HD-PTP is a 180KDa multidomain protein that contains a **Bro1** domain, a **coiled-coil** domain, a Pro-rich region (**PRR**), and a C-terminal **PTP** domain. Bro1 binds STAM2 and CHMP4, subunits of ESCRT-0 and –III respectively. The colied-coil domain interacts with UBAP1 of the ESCRT-I complex. Bro1 and coiled-coil domains are also found in Alix, a protein involved in cytokinesis and viral budding. Binding to STAM2 and UBAP1 is unique to HD-PTP, but CHMP4 binds also to the Bro1 domains of human Brox (unknown function) and Alix. To understand the basis for this specific binding we have determined high resolution structures of the Bro1 and coiled-coil domains of HD-PTP in complex with several subunits of the ESCRT machinery.

The structure of the HD-PTP coiled-coil domain reveals an unexpected extended fold, different from the coiled-coil domain (V domain) in Alix, that explains the selective binding to UBAP1. The structures of the Bro1 and coiled-coil complexes revealed the details of the binding interfaces in HD-PTP at the atomic level. This data, together with biochemical binding analysis and mutagenesis have established the molecular determinants for specific interactions and selectivity between Bro1-containing proteins.

The results invoke a model in which HD-PTP, via its N-terminal domains, plays a role as a major scaffold binding platform to regulate ESCRT-dependent trafficking as well as co-localisation of other important signalling pathways.

Keywords: phosphatase, EGFR signalling, scaffold protein, ESCRT complexes

MS5-P13 Structures of ferredoxin/flavodoxin-NADP(H) oxidoreductases in *Bacillus cereus*

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Ferredoxin/flavodoxin-NADP(H) oxidoreductases (FNRs) catalyse the NADPH dependent reduction of the electron transfer mediators ferredoxin (Fd) and/or flavodoxin (Fld). FNR and Fd/Fld hence form an electron transfer network for efficient shuttling of electrons to different Fd/Fld-dependent enzyme systems.

Three flavodoxins, two ferredoxins and three ferredoxin/flavodoxin reductases have been identified in *Bacillus cereus*, but it is so far not known how they interact. We have previously, using a tag-free purification procedure, purified and crystallised several of these electron transfer proteins and also some of their redox partner enzymes. Here we present the structures of two of the ferredoxin/flavodoxin-NADP(H) oxidoreductases from *Bacillus cereus*.

Further investigations will focus on protein-protein complexes of the electron transfer proteins. The aim is to establish whether there are any specific interactions between the proteins, *i.e.* whether any one protein preferably interacts with any of its redox partners, and if so, what the structural basis is for this interaction.

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Keywords: protein crystallography, redox enzymes, flavoproteins