

describe the structure of a new class of CBP from the parapoxvirus, Orf virus. The crystals of this protein were challenging to produce and optimized significantly through the use of somewhat surprising additives. Crystals occupy Space Group P6₅22 with unit cell parameters of $a = b = 75.62$, $c = 282.49$ Å, $\alpha = 90$, $\beta = 90$, $\gamma = 120^\circ$. The structure was phased using MAD methodologies and currently the 2.1Å structure is undergoing refinement. Early analysis indicates that it is a member of the β -sandwich family but it is quite distinct from other family members when superimposed. Additionally the crystal structure is consistent with a physiologic dimer and displays a very broad β sheet on its surface containing contributions from more than 10 β strands. The dimeric nature of this CBP appears to be a unique property of its class and may be key in explaining how it is able to bind different chemokines from at least two distinct chemokine classes.

Keywords: protein crystallography, chemokine, virology

FA1-MS11-P04

How to minimize X-ray dose used for in-house data collection on protein crystals? Vernon Smith^a,

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Datasets for *de-novo* structure solution are typically collected at synchrotron beamlines. However, with increasingly bright rotating anode generators, a significant number of datasets are collected in-house. Any exposure to X-ray radiation create free radicals inside the crystal, which can lead to decreased resolution, decreased $\langle I/\sigma(I) \rangle$, increased mosaicity and increased B-factors. It is clear that radiation damage does occur during longer data collections on rotating anode sources, even at 100 K. Unnecessary radiation damage can be avoided by using a system which maximizes detection of diffracted X-ray photons enabling more conservative incident X-ray doses to be inflicted.

The latest generation of microfocus sealed-tube sources deliver an incident X-ray beam of greater intensity than traditional rotating anode generators, but with much better beam properties and stability. Coupling with a high-sensitivity, low-noise detector creates a system (Figure 1) capable of measuring high-quality datasets while minimizing data deterioration through radiation damage.

An example will be presented comparing data from the newly developed solution with data obtained using a 'classical' rotating anode-imaging plate combination. Special attention will be paid on the X-ray dose the investigated sample receives during the data collection.

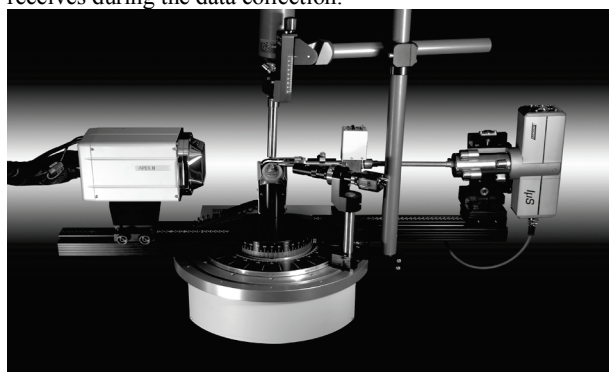


Figure 1: In-house system for X-ray dose minimization: X8 PROSPECTOR

Keywords: X-ray dose, microfocus source, crystal damage

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Crystal structure of SppB_{TK}, a putative signal peptide peptidase from *Thermococcus kodakaraensis*.

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Proteins secreted by the Sec-dependent pathway contain N-terminal signal peptides that are cleaved by signal peptidases following transport. The remnant signal peptides are then degraded by membrane-bound signal peptide peptidases (Spp). The crystal structure of the soluble domain of SppA from *E. coli* (SppA_{EC}, 67 kDa) showed that this protein consists of two domains with nearly identical structures, which assemble into a tetrameric ring [1]. *Thermococcus kodakaraensis* is a hyperthermophilic archaeon that possesses a Spp gene (SppA_{TK}) with approximately half the size of SppA_{EC} (36 kDa) and is most homologous to the C-terminal half of SppA_{EC} [2]. In addition, it also possesses another putative Spp gene (Tk0130), encoding a protein (SppB_{TK}) with 18% homology to SppA_{TK}. Biochemical data suggest that this protein functions as a signal peptide peptidase. Here, we present the crystal structure of the soluble domain of SppB_{TK} in the free and substrate-bound forms. SppB_{TK} structure is homologous to ATP-dependent protease ClpP and the C-terminal half of SppA_{EC}. It is an oligomeric protease assembled into an octameric ring. The active site of SppB_{TK} consists of Ser₁₃₀-His₂₂₆-Asp₁₅₄ triad, different from the Ser-Lys dyad of SppA_{TK} and SppA_{EC}. Co-crystallization of S130A-SppB_{TK} with a tetrapeptide substrate revealed the substrate binding mechanism of the protein. Based on these results, we discuss about the possible role of SppB_{TK} in signal peptide degradation in archaea.

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Crystal structure of AsaP1 metalloendopeptidase in complex with its propeptide. Xenia Bogdanović^a,

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