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Structural Studies on MsmE, a Sugar Binding Lipoprotein from *S. pneumoniae*

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Streptococcal organisms share a well-conserved, binding protein dependent, Multiple Sugar Metabolism (msm) system which is responsible for the transportation and metabolism of multiple sugars. This system is comprised of 8 proteins including 2 membrane proteins (MsmF and MsmG), an ATP-binding protein (MsmK) and the 45KDa sugar-binding lipoprotein MsmE [1].

Both native and selenomethionine derivatised MsmE were crystallised in space group $P6_122$. Selenomethionine derivative crystals showed diffraction to 2.5Å and 3-wavelength MAD data were collected at SRS, Daresbury. Selenium sites (19/22) were found using SnB [2] with peak data and site refinement and phasing using Sharp/AutoSharp [3] using MAD data along with a 3Å native dataset.

The initial model shows a two domain periplasmic binding protein-like fold and model-building and refinement is continuing.

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Keywords: MsmE, crystallography, selenomethionine derivatives

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Macromolecular Crystallography at Room Temperature: Wavelength Dependence Radiation Sensitivity and Damage

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Biological samples are known to be strongly radiation sensitive and suffer from radiation damage during room temperature X-ray data collection even at cryogenic temperatures (100K). One of the factors affecting the rate of radiation damage on biological samples is mainly to use of the wavelength for 3D structural analysis. Here we attempt to determine the wavelength dependence effects of radiation on biological samples. Our results show that radiation sensitivity presents a direct dependence with the wavelength. Several derivatives from Hen Egg White Lysozyme crystals were grown in the gel by the Counter Diffusion Method in standard Hampton Research Granada boxes. Synchrotron radiation data were recorded at the X6A beam line of the National Synchrotron Light Source at room temperature. A threshold wavelength was determined for each sample derivative for which radiation sensitivity and damage significantly increased.

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Keywords: macromolecular crystallography, radiation damage, wavelength

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Solution Scattering Studies of Xylanase XYNII from *Trichoderma longibrachiatum*

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Xylanase (endo-1,4-β-xylan xylanohydrolase, EC 3.2.1.8) is an enzyme catalyzing the hydrolysis of β-1,4-D-xylosidic linkages of xylan. *Trichoderma longibrachiatum* has two different xylanases: XYNI and XYNII. Xylanase XYNII is a small protein (21 kDa; 190 a.a).

X-ray solution scattering measurements of XYNII were performed on the EMBL X33 beam line at DESY, Hamburg (Germany). The SAXS/WAXS camera was used to cover the scattering vectors $0.16 < s < 9.2$ nm^{-1}, with $s = 4\pi\sin\theta/\lambda$, where the 2θ is the scattering angle and $\lambda = 0.15$ nm. The radius of gyration, forward scattering and distance distribution functions were calculated using the program GNOM [1]. The low resolution structure was restored from experimental data by programs: DAMMIN [2] and GASBOR [3].

The radii of gyration R_G measured by SAXS were 1.65 nm (pH 3.0) to 1.72 nm (pH 10). The experimental scattering curve was compared to this evaluated from crystal structure. The low resolution structure and also the domain structure (chain compatible spatial distribution model) will be presented.

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Keywords: small-angle X-ray scattering, wide-angle scattering, xylanase

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Towards the Structure Determination of the HtrA1 Protein from *Staphylococcus aureus*

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The HtrA1 protein of *Staphylococcus aureus* has a high degree of sequence homology to members of the HtrA/DegP family from Gram-negative bacteria. Expression of this protein is induced in response to heat shock or secretion stress signals in *Bacillus subtilis* [1]. HtrA/DegP shows a temperature dependent "switch" from chaperone to (serine)protease activity [2], a function that has also been proposed for the corresponding protein in Gram-positive bacteria [3]. We are expressing HtrA1 with a view to crystallization and structure determination. A His-tagged wildtype HtrA1 protein was expressed, containing an enterokinase cleavage site in a flexible linker region. Initial purification revealed that the protein probably undergoes self-cleavage during removal of the His-tag. For this reason the serine residue of the proteolytic site was mutated to alanine. This mutant protein was purified and appears stable during the process of enterokinase clevage and subsequent purification. Using size exclusion chromatography the HtrA1(SA) protein shows an elution profile corresponding to a monomeric molecule. Crystallization trials are currently under way, and promising conditions have been determined.

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Advanced High-throughput Platforms for Protein Crystallography

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In recent years, the area of protein crystallization has been subject to fundamental developments. The demand for sophisticated and diversified platforms, especially with regard to optical properties, multiple screening capabilities, and suitability for small sample volumes, has resulted in the creation of highly specialized, multi-faceted products to meet the diverse requirements of automated high-throughput approaches. Combined with additional necessities for more