like a tetramer. Ultracentrifugation and native PAGE results also do not conclusively show whether it is a trimer or tetramer [4]. Initial crystallographic data however showed 3 molecules in the asymmetric unit suggesting an elongated trimer. SAXS scattering curve of the IgD binding domain is indicative of a trimeric arrangement and *ab initio* modeling confirms fibrous elongated shape. Recently circular dichroism spectroscopy was done on MID962-1200 and the data were deconvoluted using DICHROWEB [5]. The analyzed data with good NRMSD value shows approximately 11% alpha helices, 32% beta sheets and 30% unordered secondary structure.

We crystallized the protein MID962-1200 with His-tag at concentration 10 mg/ml, but had problem with reproducibility limiting our ability for experimental phasing.Now we have recloned it in pETM-30 with a cleavable GST-tag, which is digested by TEV protease [6] made in house, to allow for crystallization of the protein without any tag. The crystal structure of MID962-1200 domain alone and in complex with its partners like IgD will elucidate the mechanism of this protein and give information on host-pathogen interactions.

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Keywords: autotransporter, elongated, dichroism

MS36.P31

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Elucidating the functions of Key regulators in biofilm formation and dispersal

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Biofilms are complex communities of bacteria that are encased in an extracellular matrix and adhere to almost any surface. They are also responsible for more than 65–80% of human infections. Moreover, these infections are extremely difficult to treat because biofilms are both highly resistant to host defenses and antibiotics. Currently, a detailed understanding of how biofilms assemble, how they are regulated at a molecular level, and how they achieve antibiotic resistance is only rudimentarily understood. Recent microarray studies have identified many of the genes that are up and down regulated in *E. coli* biofilm formation. We are using X-ray crystallography, combined with genetic and biochemical experiments, to determine the function of these proteins in order to understand their roles in biofilm formation and stability. Here, we report the expression, purification, crystallization and structures of two of these biofilm proteins, one which mediates biofilm dispersal (2.0 Å) and a second which directs biofilm formation (2.8 Å). Complementary genetic and biochemical experiments (electrophoretic mobility shift assays and isothermal thermal calorimetry) using the structural information as a guide are now being used to elucidate their *in vivo* ligands and functions. These studies are providing novel insights into the protein products that drive biofilm formation, dispersal and stability which, in turn, can be used as targets for the development of novel drugs to treat biofilms in the environment and disease.

Keywords: biofilm, E. coli

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Crystal structure of *S.aureus* AtlE homologous to the glucosaminidase domain of major AtlA

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Autolysins are a diverse group of enzymes responsible for degradation of peptidoglycans forming the bacterial cell wall. They are involved in a number of cellular processes including the cell wall expansion and cell division. They are also implicated in the bacterial pathogenesis. It was shown that autolysin deficient mutants of many bacterial strains exhibit lower virulence than their parental wild-type strains.

Methicillin-resistant strain of *Staphylococcus aureus* (MRSA) is a multidrug-resistant bacterium responsible for several difficult-to-treat infections in humans. The major autolysin A (AtlA) is the predominant autolysin in *Staphylococcus aureus*. It consists of N-terminal aminidase domain followed by the three cell-wall binding repeats and the C-terminal glucosaminidase domain. The genome of *Staphylococcus aureus*, however, contains additional autolysins. Here we present the crystal structure of Staphylococcus aureus autolysin E (AtlE) which exhibits high similarity to the glucosaminidase domain of AtlA. AtlE adopts a heart like fold. Despite no amino acid sequence homology between the AtlE and lysosome, the central helical core of AtlE alignes to the core structure of lysozyme. The two structurally unique subdomains of AtlE located at the top left and right side of the core domain additionally expand the structure. At the interface of both domains a deep and extended active site cleft is formed with a number of conserved Asp and Glu residues.

Functional characterisation of AtlE showed that the enzyme exhibits cell wall degrading activity and stimulates the formation and growth of biofilms. Since many infections caused by Staphylococcus aureus appear to be associated with biofilms, the AtlE structure may assist in the development of novel antibiotics.

Keywords: autolysin, glucosaminidase, biofilm

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Acoustically mounted microcrystals yield high resolution X-Ray structures

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Microcrystals measuring only a few microns along an edge are often easy to obtain but difficult to use because they are too small to yield a suitable diffraction pattern with conventional macromolecular crystallography (MX). Fortunately, advances in X-ray sources at third generation synchrotrons and free electron lasers (FEL) are rapidly reducing the sample size and exposure time required for atomic level crystal structure determination. However, as the crystal size is reduced, so is the signal relative to the noise in the X-ray diffraction data. Consequently, an essential strategy to improve the signal to noise ratio is to reduce the background scattering, especially from the mother liquor surrounding a micron-sized crystal. Robust new strategies must be developed to manipulate microcrystals for structure determination.

To address this critical gap, we are developing acoustic droplet

ejection (ADE) methods to accurately and gently transfer small protein crystals (roughly 10 μm on each side) within microdroplets of mother liquor from the crystallization well, through a short air column (1-10 cm), to a standard X-ray diffraction mounting mesh. The acoustic droplet ejection instrumentation uses sound energy to transfer nanoliter to picoliter volumes from the surface of liquids. The successful use of ADE to transfer living cells and isolated DNA without inducing strand breaks suggests that it might be gentle enough for protein crystals.

Here, we report that ADE methods are well-suited for transferring 2.5 nanoliter droplets of microcrystal slurries of insulin or lysozyme from a 384-well plate to standard MiTeGen™ (Ithaca, NY) micromesh mounting pins. After cryocooling, the micron-sized crystals are located on the mesh with the X-ray beam via a rasterscan strategy by the presence or absence of diffraction. Once microcrystals are located, partial datasets are collected and crystal structures solved to better than 2 Å resolution from merged datasets. Importantly, high resolution structures can be solved from slurries of microcrystals that traditionally would have been discarded as unsuitable for X-ray diffraction studies.

Keywords: microdiffraction, method, synchrotron

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The use of kinoform lenses as an option for microbeams in macromolecular crystallography

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Most new generation macromolecular beam lines offer small beams in the tenth of micron range. Significant reductions in beam size imply in significant beam flux losses. The inclusion of kinoform lenses in the existing beam line optics may be a simple and cheap alternative to obtain beams in the 1 micron range.

A kinoform lens designed to focus in the one-to-one configuration was inserted in the beam path of the bending magnet beam line X6A at the National Synchrotron Light Source. The optical design for the X6A beam line is very simple, with a channel cut Si (111) monochromator and a toroidal focusing mirror, the focused beam size on the sample is of the order of several hundred microns. Using the image of a precision adjustable slit 1m upstream of the sample position the kinoform lens allowed us to produce a beam size that is adjustable from the smallest measured size of 20 microns up to the size normally produced by the beamline optics. Advantages of this approach are 1) an improved signal to noise, 2) a conveniently adjustable trade-off between spot size and flux on sample, and 3) simple configuration change from small beam mode to the normal, larger beam size mode.

Keywords: kinoform lens, macromolecular crystallography

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Protein micro-crystallography at the micro focus beamline BL32XU at SPring-8

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A newly developed micro-focus beamline, BL32XU at SPring-8, is dedicated to the protein-micro crystallography[1]. Available focused beam size is from 1 to 10 μm square with photon flux density of 10^{10} phs/sec/μm^2. The user operation of this beamline started from May 2010 for the domestic users, and now opens its 20% beam time for public user worldwide. The beamline is operated mainly for the National Project, named "Targeted Proteins Research Program".

Structure determinations of proteins are often hindered when the size of available crystals is small, even though using the synchrotron radiation. However, proteins involved in recent target such as membrane proteins or protein complexes, tend not to grow largely enough for providing good diffraction signals. Thus, demands for achieving protein micro-crystallography are getting larger. Accordingly, the construction of this beamline was started from 2007 at SPring-8.

We had successfully completed the commissioning of the beamline at the end of 2009. The achieved beam size at sample position corresponded to 0.9 x 0.9 μm^2 with 6 x 10^{10} photons. The beam size is easily changeable by users from 1 to 10 μm square with the almost same flux density.

An equipped automatic sample changer, SPACE[2], can mount so-called Hampton-style pins stored in UNIPUCK trays[3]. The robot enabled user to conduct beamline experiments completely from outside of the hutch[4]. This is also important for stabilizing a position of the micro-beam against the temperature change of the hutch inside. By keeping the hutch temperature precisely, the drift can be controlled below 2 μm per a day, which is easily fixed with a few minutes automatic beamline tuning. For reducing background scattering from the air, a helium chamber which sealed sample environment was developed and usable in co-operation with the helium gas cryo-cooler and SPACE which had a compact arm to access to the goniometer.

Through one year user operation of the beamline BL32XU, some experiments, previously considered to be difficult, were achieved by using its micro-beam with high flux density, such as collecting a full diffraction dataset from 3 μm protein crystal, acquiring high quality dataset from a crystal harvested from the initial crystallization condition, probing single-crystal volumes from a heterogeneous protein crystal, and so on.

These results proved that the beamline benefited users by cutting off their time to optimize crystallization conditions especially for smaller and lower quality crystals.

We will also present about the high throughput screening system of protein micro-crystals using the CMOS detector[5].

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Keywords: synchrotron, microcrystallography, protein

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The long-wavelength MX beamline I23 at diamond light source

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Experimental phasing exploiting the anomalous signal from protein or RNA/DNA crystals around specific absorption edges has become the method of choice to solve the crystallographic phase problem in macromolecular crystallography (MX) in the absence of molecular replacement models. For metallo-proteins such absorption edges are within the wavelength range from 0.6 to 2.2 Å typically provided by