

micro meter to 10 micro meter. Until now a protein crystal is picked up manually from a crystallization droplet using a cryoloop and is mounted on a goniometer head. However it seems to be impossible to manipulate the protein microcrystal by hands, because the protein microcrystals are very small and fragile against a shock. So we are developing an automatic microcrystal pick-up system. In the system, we applied optical tweezers to manipulate the fragile protein crystal. It was reported that the optical tweezers at the near-infrared region traps and manipulates a cell without critical photodamage. We tried two kinds of optics for optical tweezers. One had a condensing lens, the other had a lensed fiber probe, which focused the laser on the object. The optical tweezers with the condensing lens succeeded in trapping of protein crystals with the size in 100 micro meter range. X-ray measurement of the trapped crystals indicated that laser trap with 1064 nm wavelength hardly affected the result of X-ray structural analysis. On the other hand, the optical tweezers with the two lensed fiber probe could manipulate the protein microcrystal with lower emission power. The lensed fiber probe was smaller than former, which had an advantage to be operated in the crystallization droplets at various crystallization plates.

Keywords: protein microcrystal, optical tweezers, crystal manipulation

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A new understanding of radiation damage at cryogenic temperatures

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Radiation damage is one of the remaining bottlenecks in structural biology. In the literature many aspects of radiation damage are very well described, however the underlying mechanism responsible for the loss of diffracting power of the crystals remained unknown so far. To get a deeper insight into the processes involved we have investigated radiation damage by combining traditional X-ray data collection with small angle X-ray scattering over a broader temperature range from 5 K to 160 K. All experiments were carried out at the protein crystallography beamline X06SA at the Swiss light source (SLS) equipped with a Pilatus 6M pixel detector. For all crystals individual increase or decay rates for different quality parameters with dose were determined and analyzed as a function of temperature. Interestingly an unexpected temperature dependence for all these parameters was observed. We could identify an optimal data collection temperature, where radiation damage is reduced by about 30% compared to data collection at 100 K, where most experiments are performed today. To gain information about the length scale of the disorder phenomena we performed small angle X-ray scattering experiments on cubic insulin crystals at the same experimental conditions and temperatures as used for the data collections. Analysis of the diffuse scattering signal lead us to two different mechanisms responsible for the loss of diffracting power. We could identify hydrogen gas, formed during irradiation of biological samples with X-rays, as the main damaging agent at cryogenic temperatures. Our findings are in good agreement with observations from cryo-electron microscopy and explain the basic mechanism of radiation damage at cryogenic temperatures for the first time in a conclusive manner.

Keywords: radiation damage, cryocooled crystallography, small-angle scattering

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Crystal structure of the peptidoglycan recognition protein at 1.8 Å resolution

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The mammalian peptidoglycan recognition protein-S (PGRP-S) binds to peptidoglycans (PGNs) which are essential components of the cell wall of bacteria. The crystals of PGRP-S belong to orthorhombic space group *I*222 with $a = 87.0\text{\AA}$, $b = 01.7\text{\AA}$ and $c = 162.3\text{\AA}$ having four crystallographically independent molecules in the asymmetric unit. The structure has been determined with molecular replacement method and refined to an R_{cryst} and R_{free} factors of 0.225 and 0.247 respectively. Overall, the structures of all the four molecules are identical. The folding of PGRP-S consists of a central β -sheet with five β -strands, four parallel and one antiparallel and three α -helices. This protein fold provides two functional sites. The first of these is the PGN-binding site, located on the groove that opens on the surface in the direction opposite to the location of the N-terminus. The second site is implicated to be involved in the binding of non-PGN molecules, it also include putative N-terminal segment residues, (1-14) and helix α 2 in the extended binding. The structure reveals a novel arrangement of PGRP-S molecules in which two pairs of molecules associate to form two independent dimers. The first dimer is formed by two molecules with N-terminal segment at the interface in which non-PGN binding site is completely buried whereas the PGN-binding sites of two participating molecules are fully exposed at the opposite ends of the dimer. In the second dimer formed by another set of two molecules in which the PGN binding sites are buried at the interface while the non-PGN binding sites are located at the opposite surfaces of the dimer. This form of dimeric arrangement is unique and seems to be aimed at enhancing the capability of the protein against invading bacteria.

Keywords: innate immune system, pgrp, pattern recognition

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Structural insights into an affinity-based selection of virus-specific public T cell receptors

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To be protected against intracellular pathogens, vertebrates have developed an adaptive immune response based on a large number of T lymphocytes. Each T cell clone displays an Ig-like receptor (TCR) specific for antigens bound to Major Histocompatibility Complex (MHC) molecules, present at the Antigen Presenting Cell surface. Although the thymic selection generates a diverse naive repertoire