via diffracted weakly absorbed field (the absorption coefficient is again increased). For the explanation of the above mentioned processes the total intensity of transversed and diffracted bunches in the Darwin's table region are analyzed at different curvatures of reflecting atomic planes. It is obtained, that with reduction of the radius of curvature the total intensity at Bragg exact angle, and over all region of Darwin's table is increased, i.e. the absorption coefficient decreases. Theoretical calculations have been carried out for a quartz single crystal for several families of reflecting atomic planes. However the above mentioned effect was most brightly observed for reflecting (10-11) planes.

Keywords: X-ray attenuation coefficient, dynamical X-ray diffraction theory, crystal lattice distortion

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Portable thermal platform for optimising protein crystallisation

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The major parameters affecting protein crystallisation have been reported and some, such as pH, ionic strength, and purity of chemicals have been successfully controlled during the entire crystallisation experiment. However temperature is a parameter that still remains elusive. Several strategies have been developed to control temperature during the crystallisation process. These include the use of temperature-controlled rooms, thermostated cabinets, temperature-controlled instrumentation prototypes and modified PCR thermocyclers. To date, the current systems have the disadvantage of failing to control and record temperature changes once the sample is removed from them. These temperature fluctuations occur several times during the crystallisation experiment e.g. while inspecting the crystals under the microscope, or when selecting and mounting the crystals for X-ray diffraction. This work reviews the current state of the art in temperature control for protein crystallisation and presents an effort to actively control and record the temperature during the entire crystallisation experiment. The system presented is an electronically controlled portable temperature control platform for screening and optimising protein crystallisation. The system is designed in the form of a double height 96 well pitch microplate where five different temperatures can be screened and monitored simultaneously. The performance of the system and crystallisation results using a number of proteins will be presented.

Keywords: temperature, optimisation, crystallisation

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In situ proteolysis for protein crystallization and structure determination

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Obtaining well-diffracting crystals remains the main bottle-neck in protein crystallography. Analysis of large-scale structural genomics projects (http://targetdb.pdb.org) reports 10~15% success rate in obtaining the structure-quality crystals from a given set of purified proteins. Thus vast majority of proteins submitted to conventional crystallization trials either do not crystallize or form poor quality crystals, which can not be used for structure determination. We present here a general rescue technique, named in situ proteolysis, allowing 15~20% increase in successful crystallization. Our method is based on observation that trace amounts of protease present in the protein sample may lead to formation of hydrolyzed protein fragment prompt to crystallization. To test if addition of protease can be used as general crystallization technique we selected 55 bacterial protein samples from the pool of Midwest Centre for Structural Genomics targets. Of the 55 proteins, 20 had previously failed to crystallize and 35 had formed crystals that were unsuitable for structure determination. Addition of chymotrypsin protease to each protein sample prior crystallization trials (in 1:100 ratio) resulted in obtaining structure quality crystals for eight proteins. The Trypsin and subtilisin protease addition to the remaining protein samples allowed obtaining three additional protein structures. The analysis of eleven solved structures demonstrated that in every case the polypeptide chain forming the crystal lattice was partially degraded by protease. The relative simplicity and significant increase in successful crystallization makes this method a prominent rescue technique in protein crystallization.

Keywords: *in situ* proteolysis, protein crystallization, chymotrypsin

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In situ proteolysis for protein crystallization and structure determination

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Analysis of large-scale studies (http://targetdb.pdb.org) shows that, of all proteins that enter crystallization trials, two-thirds will not crystallize, and half of those that do crystallize can not be optimized to form suitable crystals for structure determination, for a final success rate of ~15% from purified protein to structure. Given the resources that are invested to generate a purified, concentrated protein and to perform extensive crystallization trials, this level of attrition is of considerable concern. The general applicability of in situ proteolysis to form protein crystals suitable for structure determination was tested by adding chymotrypsin or trypsin to crystal trials of a test set of 70 bacterial and human proteins that had proven recalcitrant to our best efforts at crystallization or structure determination. 13 structures were determined from this test set, which more than doubled the success rate of structure determination from purified protein. Application of the method to more bacterial and human proteins has already yielded 10 additional structures, 5 from prokaryotes and 5 from human.

Keywords: *in situ* proteolysis, protein crystallization, chymotrypsin