The biological function of proteins is dominated by hydrogen atoms. Neutron protein crystallography enables us to determine positions of hydrogen atoms, which allows rational drug design and the understanding of enzymatic processes. Although recent advances in proteins to crystallize, the bottleneck of neutron protein crystallography still remains the growth of large crystals (-1 mm^3). To overcome the difficulties in obtaining a mm-sized crystal, we have developed a new method that we call the Pendant Technique. This method has improved the Floating And Stirring Technique (FAST) and seeding techniques. As shown in Fig.1, a seed crystal was hanged in a protein solution to prevent poly-crystallization by separating additional nucleated crystals. With a long-term growth and promotion of growth speed by solution stirring, we have successfully grown a $5.9 \times 3.8 \times 3.7$ mm single hen egg-white lysozyme crystal. Using this method, we have also grown HIV protease up to $3.2 \times 1.7 \times 0.5$ mm. Therefore, this technique gives us a suggestion for production of huge crystals which are enough for neutron protein crystallography.

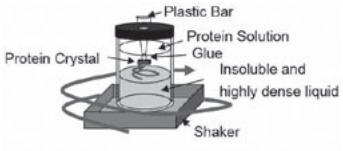

Fig.1. Schematic illustration of the method. Protein crystals can be grown hanging in solution.

Keywords: protein crystallization, crystallization methods, neutron crystallography

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Crystallization and biochemical analysis of eIFA protein

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eIFA(eIF4GI analogue) participates in nonsense mediated mRNA decay (NMD) which controls mRNA quality in eukaryote. Although down-regulating of eIFA is thought to abrogate NMD, its function in NMD is not clear yet. And eIFA is found to interact with CBP80, a member of heterodimeric nuclear cap-binding complex (CBC). CBC binds to 5' cap of mRNA and then involves in mRNA modification and elimination. eIFA protein fused with 6-His tag was expressed in E.coli and purified with affinity, ion exchange and gel filtration column chromatography. The crystals of eIFA were obtained at 290K by microbatch method. Biochemical assay on translation suggests that eIFA has a direct role in initiation of translation.

Keywords: eIFA, NMD, translation

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The scottish structural proteomics facility

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The Scottish Structural Proteomics Facility (SSPF) is a cooperative of researchers with interests in structural biology, enzyme chemistry, structure-based inhibitor discovery, virology, glycobiology and microbial pathogenesis established with funding from the Scottish Funding Council and BBSRC (UK). Our objectives are: 1. To establish a sustainable centre of excellence in Structural Proteomics. 2. To exploit post-genomic sciences and technologies to increase our understanding of pathogen physiology at the molecular level. 3. Establish a Computational Chemistry group and exploit opportunities for the rational design of novel antimicrobial agents. 4. Provide new drug leads to combat emerging multiply-resistant infectious agents. 5. Forge links with local, national and international biotechnology-pharmaceutical companies. The funding available to us is considerably less than provided to structural genomics centres in the USA, Japan or in Europe and we have adopted a focused approach to our targets closely tied to existing experience and research interests. To date we have determined numerous structures that inform about unusual aspects of enzyme chemistry, essential biosynthetic pathways in pathogens, pathogenicity, DNA repair, viral proteins and carbohydrate transport. We have established the technologies to drive computational chemistry approaches to drug discovery where a small subset of our targets is relevant. We have been testing high-throughput methods, novel expression protocols and systems. Our targets include proteins from viruses, archea, bacteria, protozoan parasites and higher eukaryotes; small cytosolic enzymes through to multi-subunit complexes and membrane bound proteins.

Keywords: structural proteomics, Scottish, drug targets

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A dynamic light scattering system combined with a conventional chromatography for sample preparation

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Recent progress in structure determination of proteins by x-ray crystallography allows us to understand the important and complicated mechanism of biological reaction in vivo. However, crystallization process of protein molecules is still the rate determining step caused by difficulty and uncertainty of the sample preparation. Measurement of dynamic light scattering (DLS) is well known as an effective method for evaluation of the sample quality, because DLS results show good correlation between dispersity of the protein solution and possibility of crystallization in many cases. However, it takes a couple of minutes for each samples to measure the polydispersity of molecules, and also, the measurements must be performed in batch mode. If a real-time DLS system is equipped to a conventional chromatography system as a detector for polydispersity of each peak, it is quite useful for evaluation of sample quality. It can be possible to recognize which fraction should be used for crystallization. Therefore, we developed a flow-type cell for a real-time DLS system which can be used as an on-line detector system for a conventional chromatography during the preparation and purification of protein samples. In this study, we report the results of the DLS measurement of alpha amylase from *Aspergillus oryzae* and other proteins by using FPLC system equipped with the real-time DLS system with the flow-type cell.

Keywords: dynamic light scattering, protein purification crystallization, protein crystallization development