Anaerobic crystallization for protein crystallography

Miki Senda	Japan	Photon Factory, High Energy Accelerator Research Organization (KEK)
Takeru Hayashi	Japan	Graduate School of Medicine, The University of Tokyo
Masanori Hatakeyama	Japan	Graduate School of Medicine, The University of Tokyo
Toshiya Senda	Japan	Photon Factory, High Energy Accelerator Research Organization (KEK)

While we can use crystallization robots and commercial-available screening-kits for protein crystallization, crystallization remains a bottleneck for protein crystallography. In general, there are two major problems. First, how to find crystallization conditions from the results of the initial screening when the initial screening gives no crystals. Second, how to improve reproducibility of crystallization; there are several factors hampering reproducibility of the crystallization. To overcome these problems, we have proposed some crystallization techniques (ACA 2016, ACA2015). In this presentation, we would like to propose an anaerobic crystallization method to address these problems. Oxidation of the protein and crystallization reagents during the crystallization causes some problems. The protein oxidization would lead to a loss of the protein homogeneity, causing a failure of crystallization. In addition, the oxidation of the protein would reduce the protein concentration in crystallization drops *via* formation of oxidation films of the protein. These are frequent problems in protein crystallization. To prevent these problems, we have developed a system for the anaerobic crystallization. In the anaerobic crystallization, all crystallization experiments should be performed in an anaerobic chamber (Anaerobic 'HARD', Hirasawa). Our test experiments of the anaerobic crystallization with various proteins demonstrated clear differences between anaerobic and aerobic conditions. We will report some of recent successful examples of the anaerobic crystallization. The SHP2_SH2 domain in complex with the EPIYA-D peptide could be crystallized under anaerobic conditions; while anaerobic crystallization gave thick crystals with high reproducibility, only very thin needle crystals were obtained with low reproducibility under aerobic conditions.