Protein crystallisation - tricks and practise

Lubica Urbanikova

Institute of Molecular Biology, Slovak Academy of Sciences, Dubravska cesta 21, 845 51 Bratislava, Slovak Republic

lubica.urbanikova@savba.sk

Preparation of highly ordered protein crystals is essential for using X-ray crystallography technique, which is still the most powerful tool for determining the three-dimensional structure. At high technical level of data collection at synchrotron sources and software developments for data processing and structure determination and refinement, protein crystallization still remains more art than science and represents one of the major limiting steps. Conditions at which a protein might produce highly ordered crystals cannot be computed, they must be identified empirically. Crystallization is influenced by extremely large number of parameters from which the most important is protein itself. It is well known that subtle changes in conditions as well as protein modifications may have a dramatic effect on crystallization behaviour.

We have crystallized a number of proteins add determined their tertiary structures. The main objects were mutants of ribonuclease Sa from bacteria *Streptomyces aureofaciens*, glucoamylases and their mutants from the yeast *Saccharomycopsis fibuligera*, actin-binding domain of cytoskeletal protein plectin from *Mus musculus*, CE16 acetylesterase from fungi *Trichoderma reesei*, GH30 xylanase A from bacteria *Dickeya chrysanthemi* and other enzymes. Successful crystallization was conditioned by the use of special approach tailored to each protein or mutant. Similarly, the choice of cryoprotectant and optimisation of the crystal flash cooling procedure was crucial for collecting good sets of data. The tested tricks and successfully used approaches will be described, illustrated and discussed.

The work was supported by the Slovak Academy Research Grant Agency VEGA grant No. 2/0190/14 and Slovak Research and Development Agency grant APVV-0602-12. We acknowledge the EMB c/o DESY, Hamburg and MFPL Vienna University for providing us with synchrotron source and laboratory X-ray source facilities.