s7.m4.o1 **How much have systematic studies on protein crystallization influenced current practices in protein crystallography?** M. Budayova-Spano & J.C. Fontecilla-Camps. *Laboratoire de Cristallographie et de Cristallogenèse des Protéines; Institut de Biology Structurale 'Jean-Pierre Ebel' CEA-CNRS-UFJ, 41, rue Jules Horowitz, 38027 Grenoble, Cedex 1, France*
Keywords: instrumentation, techniques for crystallisation.

In 1985, the first international meeting on protein crystal growth (PCG) took place in Stanford University. There, and for the first time, protein crystallographers and crystallizers, and scientists from the small molecule crystallization community were brought together to discuss possible applications of the knowledge accumulated by the latter to solve the problems of macromolecular crystallization. In spite of a very promissing beginning, fifteen years later it is clear that many of the very powerful ideas and methods proposed then (and still being developed) have not become widely used in protein crystallography laboratories. As an example, in the period 1960-1998, there were only about 50 successful temperature-controlled PCG experiments reported, as compared to over 2000 constant-temperature vapor diffusion-based results. And this in spite of the fact that temperature is considered to be a very effective precipitating agent for small molecules and its use has been strongly recommended in the case of proteins.

The popularity of vapor diffusion (vp) approaches, especially the hanging drop method (75 % of vp experiments) may be explained by its low cost, the requirement of small amounts of protein, and the ease with which the method can be implemented when using either incomplete factorial or sparse-matrix strategies. The latter approach has become increasingly popular because is now commercially available and does not require any decisions to be made by the experimentalist. Any positive result may subsequently be refined using a systematic approach exploring increasingly finer conditions. One of the major problems when assessing the effectiveness of a given crystallization approach is that negative results are almost never reported in any detail. The large number of cases where the proteins have been modified (proteolysis, deglycosylation, site-directed mutation, etc.) clearly indicates that, when crystallization is not readily attained, rather than modifying the crystallization strategy, scientists prefer to modify the sample.

At the conference we will give an overall assessment of the various chemical and physico-chemical methods being used to improve our understanding of PCG and maximizing success in obtaining well-diffracting crystals. We will also attempt to determine which of these approaches could be of immediate benefit to the standard protein crystallography laboratory and which may be concentrated in national and/or international facilities to deal with the really difficult cases.

s7.m4.o2 **Novel Cryo-cooling techniques.** S. Panjikar, A. Riboldi-Tunnicliffe and R. Hilgenfeld, *Institute of Molecular Biotechnology, Department of Structural Biology and Crystallography, Beutenbergstr. 11, D-07745, Jena, Germany, panjikar@imb-jena.de, atunni@imb-jena.de, hilgenfd@imb-jena.de*
Keywords: instrumentation, techniques for crystallisation.

Among the biggest problems in macromolecular crystallography is the relatively weak diffraction power of the crystals and their sensitivity to ionizing radiation. Therefore, the use of low temperature methods offers a number of advantages. Of primary pracitcal importance is the decrease in X-ray damage to the crystal. This is especially important for crystals which require synchrotron radiation for data collection (e.g., MAD experiments).

The current method in low-temperature crystallography of biological macromolecules is the introduction of a cryoprotectant solution into the crystal, in order to prevent ice formation. This method has generally proven useful but requires a time- and crystal-consuming search for a suitable cryoprotectant and occassionally fails altogether. Also, it is sometimes observed that the cryoprotectant removes weakly bound ligands from the protein under study.

We have therefore developed novel methods to prevent ice formation which have completely removed the usage of conventional cryoprotectants in our laboratory. One of these employs immissible paraffin oil [1], and the other a rather unconventional cryoprotectant which we call Panjelly™. Due to the simplicity of these methods, we would like to argue that they be tried as first choice in cryocrystallography, since they do not require the growth or soaking of crystals in solvents which could disturb the packing by diffusing into the crystal. Also, we have found that crystal reannealing is much more efficient when combined with paraffin oil. However, the ultimate method seems to be a combined treatment using Panjelly™ for flash-cooling and the oil in a single subsequent annealing step.

Also, Panjelly™ is found to give a much improved diffraction limit of the crystals even prior to reannealing. We will describe several cases where the maximum achievable resolution was around 8 Å when conventional cryoprotectants were used, and 3 Å when we applied Panjelly™ and paraffin oil. In these cases, the usage of these novel cryoprotectants actually allowed continuation of the project when it would otherwise have had to be given up.

[1] A. Riboldi-Tunnicliffe and R. Hilgenfeld, J. Appl. Cryst. 32 (1999) 1003-1005.