MS01 WHEN BAD CRYSTALS TURN GOOD: TRANSFORMING POORLY DIFFRACTING CRYSTALS
Chairpersons: Naomi Chayen**,** Janet Newman

MS01.24.1
Acta Cryst. (2005). A**61**, C9
Optimizing Your Crystals—Is it Really Worth the Time?
Annie Hassell, *Computational, Analytical & Structural Sciences Dept., GlaxoSmithKline, Research Triangle Park, North Carolina 27709 U.S.A.* E-mail: annie.m.hassell@gsk.comF

With the advent of the Structural Genomics initiatives, we have seen a host of new technologies emerge for automation of the tedious crystallization process. The latest robots and nanodispensers allow us to perform a larger number of crystallization screens faster with significantly less quantities of protein. There is a great temptation to harvest crystals directly from these small drops and immediately collect a data set---without any further improvement of the crystal quality. While this works well in some instances, there are many crystals that require further optimization before they are useful for structure determination.

Optimization strategies will be presented for a variety of different proteins in the following areas:

- Protein preparation
- Crystallization--methods and conditions
- Cryocrystallography—cryosolvent selection, soaking tricks

Keywords: crystallization of proteins, crystallization methods, optimization

MS01.24.2
Acta Cryst. (2005). A**61**, C9
Improvement of Crystal Quality by Time Controlled Annealing
Vivian Stojanoff[a], Ardeschir Vahedi-Faridi[b], Joanne I. Yeh[b], [a]*BNL-National Synchrotron Light Source, Upton, USA.* [b]*Brown University, Department of Molecular Biology, Providence, USA.* E-mail: stojanof@bnl.gov

Poorly diffracting crystals is one of many bottlenecks affecting bio-molecular X-ray crystallography. Different groups have reported on the development of procedures and techniques to improve the diffraction quality of bio-molecular crystals. These procedures, generally labeled as “annealing”, remain mostly subjective and anecdotal. To systematically determine the effect of flash-cooling on mosaicity and intensity a small device was developed at the NIGMS facility at the NSLS that allows time controlled annealing. Repeated annealing of glycerol kinase crystals revealed significant changes in the diffraction pattern: position and intensity distribution of individual reflections. The results showed that repeated flash-annealing cycles cause a significant decrease in the overall averaged mosaicity together with an increase in the measured maximum intensity and an enhanced signal to noise ratio. Changes in individual reflection profiles will be discussed in light of domain structures and defect analysis.

Acknowledgements: We would like to thank A. Lenhard, B. Clay and Z. Yin for their contribution in the development of the annealing device. Funding for this work was provided by the NIGMS, Y1 GM-0080 and the U.S. Department of Energy, contract #DE-AC02-98CH10886. AVF acknowledges the financial support of the National Institute of Health.
Keywords: crystal quality, flash-cooling, mosaicity

MS01.24.3
Acta Cryst. (2005). A**61**, C9
Designed Ankyrin Repeat Proteins as Tools for the Crystallization of Proteins
Markus G. Grütter, *Department of Biochemistry, University of Zurich, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland.* E-mail: Gruetter@bioc.unizh.ch

Ankyrin repeat proteins (ARPs) are ubiquitous protein-protein interaction molecules fundamental to many biological processes. By consensus sequence and structure analyses of ARPs, we derived a repeat module of 33 amino acids with fixed framework residues and randomized surface residues suitable for target binding. The random assembly of such modules yields combinatorial libraries of naive ARP’s of varying length and diversities larger than 10^{10}. Unselected library members are well expressed and stable and show the correct fold [1]. Using ribosome display we selected specific binders against different protein targets with affinities in the low nanomolar range [2]. This opens the possibility to crystallize a target protein in complex with ARPs and enhances the chances of obtaining structures of target proteins difficult to crystallize. We have applied this technology to a variety of different proteins such as proteases, kinases and membrane proteins. The methodology and structures of unselected ARP’s alone as well as of an ARP-maltose binding protein complex and an ARP-kinase complex will be presented proving the usefulness of selected ARP’s in structural biology. The technology opens a new avenue in macromolecular crystallization and is an attractive alternative to antibodies in the crystallization of membrane proteins.

[1] Kohl A., Binz H.K., Forrer P., Stumpp M.T., Plückthun A., Grütter M.G., *PNAS*, 2003, **100**, 1700-05. [2] Binz H. K., Amstutz P., Kohl A., Stumpp M.T., Briand C., Forrer P., Grütter M.G., Plückthun A., *Nature Biotech*, 2004, **22**, 575-82.
Keywords: repeat proteins, crystallization, complexes

MS01.24.4
Acta Cryst. (2005). A**61**, C9
Effect of Crystal Size and Cooling Method on Cryoprotection and Data Quality
Constance Schall[a], Unmesh Chinte[a], Binal Shah[a], Leif Hanson[b], [a]*Department of Chemical and Environmental Engineering, University of Toledo.* [b]*Instrumentation Center, University of Toledo, Toledo, USA.* E-mail: cschall@eng.utoledo.edu

D-xylose isomerase crystals, with glycerol as cryoprotectant, were flash cooled in the cold nitrogen gas of an Oxford 700 Series Cryostream at 100 K and by plunging in liquid nitrogen. X-ray diffraction data were measured with a Saturn CCD on a Rigaku FR-E X-ray source, processed with Rigaku’s CrystalClear 1.3.6 software and crystal quality was assessed at various glycerol concentrations. The minimum glycerol required to successfully flash cool crystals of variable size was determined. The glycerol requirement was found to be a strong function of crystal size. This agrees with our conclusions of earlier studies using different size loops with glycerol added to Hampton Screen solutions (J. Appl. Cryst., in press). Comparing the results obtained with gas cooling with those obtained by plunging in liquid nitrogen suggests that liquid nitrogen does not give significant improvement in cooling rates as expected. This is most likely due to film boiling. In general, data quality of gas cooled crystals was better than that of liquid plunged crystals. Comparisons also were made using a ‘slush’ of partially frozen nitrogen. Early experiments with nitrogen slush suggest faster cooling rates as compared to those obtained with liquid and gaseous nitrogen. Large crystals were soaked in glycerol solutions for different times to determine minimum soak time required for near complete diffusion of cryoprotectant solutions. This time can be estimated through a simple calculation of a ‘penetration’ time. The soak time was found to have a significant effect on success of flash cooling and quality of diffraction data.
Keywords: flash cooling, crystal size, cryoprotectant

MS01.24.5
Acta Cryst. (2005). A**61**, C9-C10
Data Mining and Machine Learning for Improved Crystallization Success - Expectations and Reality
Bernhard Rupp, *University of California - LLNL, L-448, POB 808, Livermore, CA 94551, USA.* E-mail: br@llnl.gov

Protein crystallization has traditionally been viewed as an art, with the outcome largely dependent on the skills (or superstitions) of the experimenter and a good portion of luck. Many competing 'recipes' for improvement include largely anecdotal and singular evidence, praying on the desperation of the unlucky experimenter.

The advance of automated protein crystallization methods over the past several years now provides the opportunity to amass substantial amounts of crystallization data. Direct capture of all experimental conditions and outcomes - including negatives - into relational data