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

MS01.24.1
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Optimizing Your Crystals—Is it Really Worth the Time?
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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

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Improvement of Crystal Quality by Time Controlled Annealing
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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.

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Keywords: crystal quality, flash-cooling, mosaicity

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Designed Ankyrin Repeat Proteins as Tools for the Crystallization of Proteins
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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

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Effect of Crystal Size and Cooling Method on Cryoprotection and Data Quality
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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

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Data Mining and Machine Learning for Improved Crystallization Success - Expectations and Reality
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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

bases should in principle allow data mining and machine learning in the hope to unearth statistically valid knowledge about how to select and optimize the best crystallization conditions for a given protein.

Indications have emerged that the process of knowledge-based predictions in protein crystallization is not going as smoothly as one would hope. The foremost reason lies in the complex and locally determined nature of the crystallization process, and the high dimensionality and sparse sampling of the multivariate crystallization parameter space [1]. Optimal experimental design, careful annotation, and robust machine learning methods have provided various reliable general rules - often affirming prior empirical suggestions - while specific predictions yet remain of limited statistical significance due to low confidence of the derived rules.

[1] Rupp B., Wang J., *Methods*, 2004, **34**, 390-407

Keywords: crystallization, data mining, predictive models

MS02 CHAMELEON PROTEINS

Chairpersons: Lynne Regan, Paul Curmi

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The Amazing Versatility of Proteins – Structural Polymorphism and Evolution

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Structures of hydrophobic core residue mutants of the immunoglobulin binding domain B1 of streptococcal protein G (GB1), a universal model protein, were determined. Surprisingly, the oligomeric state and quaternary structure of several of these mutant proteins is drastically changed. A domain-swapped dimer and a symmetric tetramer, with inter-molecular strand-exchange involving all four units were discovered. These findings demonstrate that proteins are able to undergo substantial global rearrangements through the acquisition of very few point mutations. The domain-swapped dimer dissociated into a partially folded, monomeric species at low micromolar protein concentrations and we have characterized this monomeric, partially folded species by NMR. Extensive conformational heterogeneity for a substantial portion of the polypeptide chain exists and exchange between the conformers within the monomer ensemble on the micro- to millisecond timescale renders the majority of backbone amide resonances broadened beyond detection. Despite these extensive temporal and special fluctuations, the overall architecture of the monomeric mutant protein resembles that of wild-type GB1 and not the monomer unit of the domain-swapped dimer. Interestingly, this partially folded monomeric species seems to constitute the critical folding intermediate for amyloid fibril formation.

Our results suggest that destabilization of a monomeric protein can be compensated for by multimerization and that alternative structures (multimers or higher order oligomers) are accessible to proteins from long-lived partially folded intermediates that are capable of large scale conformational fluctuations.

Keywords: structural polymorphism, evolution, mutations

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The Mechanisms of Conversion of Proteins into Amyloid Fibrils

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In addition to folding to unique and well-defined three-dimensional structures that are relatively easy to crystallise and analyse with X-ray crystallography, proteins also have a tendency to misfold and self-assemble into stable fibrillar aggregates. These structures, known as amyloid fibrils, are responsible for over 20 human diseases including Alzheimer and Parkinson's diseases and various systemic amyloidoses.

Amyloid formation is not limited, however, to the few macromolecules associated with diseases but is a generic property of natural and synthetic proteins. An understanding of the process of amyloid formation is therefore essential not just for elucidating the pathogenesis of amyloid diseases, but also for the rational design of proteins with the ability to escape aggregation. Given the wide range of morphologies and structures that can be achieved by converting different proteins into amyloid fibrils, the rational and controlled formation of these structures can give rise to a number of materials with useful but yet unexplored properties.

I will describe the mechanism of amyloid formation of proteins, with particular emphasis on the structural and amino acid sequence determinants of this process. Experimental evidence suggests that amyloid formation follows rules that are rather general and applicable to various systems.

Keywords: amyloidogenesis, folding, protein assembly

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Structural Changes in the Bacterial Toxin Pneumolysin During Pore Formation

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The bacterial toxin pneumolysin is released as a soluble monomer that kills target cells by assembling into large oligomeric rings that form pores in cholesterol-containing membranes. Using cryo-EM and image processing we have determined the structures of both the prepore and membrane-inserted pore oligomer forms, providing a direct observation of the conformational transition into the pore form of a cholesterol-dependent cytolysin.

To form the pore structure the pneumolysin domains reorganize and double over into an arch, forming a wall that seals the bilayer around the pore. This transformation is accomplished by membrane deformation and the substantial refolding of two of the four protein domains. The pore structure supports the hypothesis that two regions of α-helices refold into β-hairpins that insert into the membrane to form the pore [1, 2]. These hairpins form the largest β-barrels observed; our largest reconstruction of the pore contains 44 subunits forming a 176 strand β-barrel around a 260 Å diameter channel.

[1] Shepard L.A., Heuck A.P., Hamman B.D., Rossjohn J., Parker M.W., Ryan K.R., Johnson, A.E., Tweten R.K., *Biochemistry*, 1998, **37**, 14563. [2] Shatursky O., Heuck A.P., Shepard L.A., Rossjohn J., Parker M.W., Johnson A.E., Tweten R.K., *Cell*, 1999, **99**, 293.

Keywords: cytolysin, toxin structure, electron microscopy

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Obeying Anfinsen: a Serpin that folds to the most Stable State

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Members of the serpin superfamily of protease inhibitors represent an exception to Anfinsen's conjecture and fold to a native metastable conformation, rather than the theoretically most stable "relaxed" conformation. Inhibitory serpins utilise metastability to inhibit target proteases. Unfortunately, as a consequence of metastability, serpins are conformationally labile, and vulnerable to mutations that promote the formation of inactive loop sheet polymers. Polymerisation of human serpins is the critical factor in the development of a number of degenerative diseases (serpinopathies).

Eukaryote serpins are sensitive to mild heating, however, to our surprise, we have identified serpins in thermophilic prokaryotes. A structural study on the serpin thermopin reveals that this molecule is able to adopt the native and cleaved state and inhibits a metastable α-