

## Crystal Growth I Macromolecular Crystallization Workshop

### MS16.01.01 SCREENING FOR CRYSTALLIZATION CONDITIONS- LESSONS LEARNED FROM 101 MACROMOLECULES. Bob Cudney, Hampton Research, Laguna Hills, CA 92653

We have examined and determined the preliminary crystallization conditions of more than one hundred proteins, peptides, and nucleic acids in our laboratory. This presentation will review this experience as an effort towards providing insight into the array of techniques one may utilize towards determining preliminary crystallization conditions of macromolecules as well as paths for the optimization.

Various sparse matrix protocols were utilized which screened a range of salts, polymers and organic solvents over a pH range of 2 to 12. Grid screens utilizing single and multiple precipitant systems of varying concentration over broad pH ranges were also utilized. Once primary crystallization variables were determined, a number of optimization screens were developed and utilized to fine tune the solution conditions for crystal growth. The protocols for these screens will be reviewed and presented.

To manage the considerable amount of crystallization data generated during experimentation, a relational database was developed and is now utilized in the design, formulation, execution, documentation, and review of crystallization experiments. With an ongoing accumulation of data from crystallization experiments, the database is becoming an increasingly important tool in the design and implementation of screening and optimization strategies while at the same time improving experimentation speed and efficiency. The features of the crystallization database will be presented, concluding with suggested areas of exploration for crystallization screening.

### MS16.01.02 POLYTHERMAL SCREENING AND PRODUCTION METHODS FOR MACROMOLECULAR CRYSTALLIZATION. George T. DeTitta, Dawn M. Dembik, Walter A. Pangborn and Joseph R. Luft. Hauptman-Woodward Medical Research Institute, 73 High Street, Buffalo, NY 14203.

Macromolecular crystal growth can be logically divided into a screening stage and a production stage. In the screening stage many or all of the parameters known to affect the outcome of a crystal growth experiment are sampled in order to identify promising candidate sets of parameters for future optimization. In the production stage the candidate sets are optimized to produce crystals of adequate size and quality for diffraction work. We are currently developing three devices based on the thermal gradient. One of these devices is optimized for the screening stage. It allows the simultaneous sampling of many conditions and requires a minimum of sample. Another is optimized for the production stage. It allows for the growth of large crystals and therefore requires larger macromolecular samples. The third device is a hybrid; it allows for both screening and production when samples are plentiful. All three employ gradients operated between 283K and 313K. Results from crystallization experiments conducted with the three devices will be discussed.

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### MS16.01.03 SALT INFLUENCE ON PROTEIN SOLUBILITY AND CRYSTALLIZATION. Arnaud Ducruix, Pascal Retailleau, Madeleine Ries-Kautt, Laboratoire d'Enzymologie et Biochimie Structurale, Bât. 34, CNRS, 91198 Gif sur Yvette cedex, France

The solubility of proteins<sup>1</sup> is related to many parameters and clearly associated to Hofmeister series<sup>2</sup>. In order to correlate ion effectiveness with "crystallizability" we selected acidic and basic model proteins and measured their solubility as a function of salt concentration. The results<sup>3,4,5</sup> suggest that the anions have their effectiveness reversed depending on the net charge of the protein, i.e. if proteins are crystallized at a pH lower or higher than their isoelectric point. As a consequence it seems that

Hofmeister series of anions are followed for acidic proteins, but reversed for basic proteins. We have used small angle X-ray scattering (SAXS) in order to correlate these observations with the influence of salts on protein-protein interactions of concentrated solutions<sup>6</sup>.

Solubility depending on the protein net charge, solubility diagrams at various pH from low to high ionic strength have been measured and will be presented. In order to assess crystal quality, we have performed preliminary experiments using the quasi-planar wave from station D25B at LURE synchrotron. Direct measurements of rocking curves from crystals of the above model proteins have shown<sup>7</sup> values as low as 1.510<sup>-3</sup> degrees.

<sup>1</sup>M. Ries-Kautt & A. Ducruix, *Methods in Enzymology*, in press.

<sup>2</sup>F. Hofmeister (1888), *Arch. Exp. Pathol. Pharmacol.*, 24, 247.

<sup>3</sup>M. Ries-Kautt & A. Ducruix (1989) *J. Biol. Chem.*, 264 745.

<sup>4</sup>C. Caronnaux, M. Ries-Kautt & A. Ducruix (1995) *Protein Science*, 4, 2123.

<sup>5</sup>J.P. Guilloteau, N. Fromage, M. Ries-Kautt, S. Reboul, F.F. Clerc, D. Faucher, D. Bocquet, C. Colonna, A. Ducruix & J. Becquart. *PROTEIN: Structure, Function and Genetics* (1996) in press.

<sup>6</sup>A. Ducruix, J.P. Guilloteau, M. Ries-Kautt, A. Tardieu, *J. Cryst. Growth*, in press.

<sup>7</sup>R. Fourme, A. Ducruix, M. Ries-Kautt, B. Capelle, (1995) *J. Synchrotron Rad.* 2, 136-142.

### MS16.01.04 IMPORTANT PARAMETERS FOR RNA CRYSTALLOGENESIS. Joseph D. Ng, Brice Felden, Gérard Keith, Philippe Dumas, Philippe Brion, Anne Dietrich-Théobald, Catherine Florentz and Richard Giegé. UPR 9002 Structure des Macromolécules Biologiques et Mécanismes de Reconnaissance, Institut de Biologie Moléculaire et Cellulaire du CNRS, 15 rue René Descartes, 67084 Strasbourg, Cedex, France

We present two model systems showing important parameters that may facilitate RNA crystal growth. First, we exemplify the importance of important RNA crystal growth features with the crystallization of a synthetic RNA minisubstrate derived from the brome mosaic virus (BMV) tRNA-like structure that was devised and synthesized to form a small RNA circle of 24 nucleotides (24cRNA). When the 24cRNA is hybridized with a 10 base complementary single-stranded RNA terminating with a CCA 3'-terminus, the RNA duplex becomes an efficient substrate for yeast histidyl-tRNA synthetase (1). This small RNA complex is arranged in a pseudoknot-like fold and is referred to as a resected pseudoknot. Using established RNA purification procedures sufficient in producing molecules for molecular biology or biochemical activity studies, small amounts of contamination, mainly of low but also of high molecular weights are still not removed. We show that these contaminants are obtained during the course of purification from electrophoretic gel matrixes or buffers revealed by NMR analysis. The elimination of these trace contaminants was critical for the successful crystallization of this molecule. Moreover, temperature was also determined to be important in crystallizing the small RNA circle such that crystals can only be obtained at 30°C or higher.

Secondly, certain additives affect positively RNA crystal growth. This is illustrated by examining a series of cationic additives which were used to study the crystallization of tRNAs. These reagents were observed to decrease nucleation and increase crystal size.

**Reference:** (1) Felden, B. (1994). Thèse de l'Université Louis Pasteur, Strasbourg.