s7.m4.o3 **Gearing Up for Structural Genomics: The Challenge of Hundreds of Proteins and Hundreds of Thousands of Crystallization Experiments Per Year.** J.R. Luft[1], J. Wolfley[1], R. Collins[1], M. Bianca[1], D. Weeks[1], I. Jurisica[2], P. Rogers[2], J. Glasgow[3], S. Fortier[3] and G.T. DeTitta[1] *(1) Hauptman-Woodward Institute, 73 High Street, Buffalo, NY 14203-1196 USA (2) University of Toronto, 140 Saint George Street, Toronto, Ontario M5S 3G6 Canada (3) Queen's University, Kingston, Ontario K7L 3N6 Canada.*
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Structural genomics promises to yield hundreds of proteins each year for structural analysis. The challenge to crystal growers is to keep pace. Our approach is to combine high throughput (HTP) crystallization setup and evaluation with sophisticated algorithmic analyses of the outcomes for the purposes of recipe prediction.

In the wet lab we have the capacity to prepare and evaluate the results of over sixty thousand (61.4K) crystallization experiments a workweek. Each is a microbatch experiment conducted under paraffin oil. Experiments are held in 1536-well micro-assay plates, each well of which contains a chemically distinct crystallization cocktail. Robotic pipetting allows the deployment of 200 nL droplets of protein stock to each of the wells of a plate in less than five minutes, allowing us to handle unstable proteins. Current total protein requirements are likely to be in the 10 mg range. After setup, plates are placed on a computer controlled XY table and translated under a digital camera where images are captured. The XY table can accommodate 28 plates (43K experiments) and the camera can record 43K images in approximately nine hours.

Images are analyzed automatically to determine the outcomes of the crystallization experiments. We are developing a standard vocabulary of outcomes that describe the results: clear drop, amorphous precipitate, phase separation, microcrystals, crystals, and uncertain outcome. These outcomes, recorded as a function of time, are the cornerstone of a crystallization database that will contain physical information about individual proteins and results of crystallization experiments. Using case-based reasoning and data mining algorithms we will identify patterns of similar properties and crystallization outcomes relating two or more proteins in the database. Our hypothesis is that, given a quantitative measure of "similarity" between proteins, recipes successfully employed for one protein will be useful starting points for crystallization experiments with similar proteins. Future work will center upon identification of the most predictive measures of "similarity" and reduction of drop volumes to 100 nL.

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s7.m4.o4 **Crystallisation of Aldose Reductase leading to Single Wavelength (0.66 A) and MAD (0.9 A) subatomic resolution studies.** E.I. Howard[1]*, R. Cachau[2], A. Mitschler[1], P. Barth[3], B. Chevrier[1], V. Lamour[1], A. Joachimiak[4], R. Sanishvili[4], M. Van Zandt[5], D. Moras[1] & A. Podjarny[1]. *[1]UPR de Biologie Structurale, 1 rue Laurent Fries, 67404 Illkirch, France [2]ABCC, NCI, SAIC, Frederick 21701, Maryland, USA [3]LCOB, ULP, 4 rue Blaise Pascal, 67008 Strasbourg Cedex, France [4]Biosciences Division/Structural Biology Center, ANL, Argonne, IL, USA [5]Institute for Diabetes Discovery, Inc., Branford, CT, USA *: Permanent address: IFLYSIB, La Plata, Argentina.*
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ALR2 (EC 1.1.1.21) is a NADPH-dependent enzyme that reduces a wide range of substrates, such as aldehydes, aldoses and corticosteroids, and it is believed to cause the development of severe degenerative complications of diabetes mellitus. Despite the large amount of biochemical and crystallographic studies done in the last decade, the fine details of the mechanism remain a subject of discussion. In order to resolve this controversy, we have pushed the structural studies to the limit of current technical possibilities. To do so, we have started X-ray crystallographic studies at the highest possible resolution, both unphased and phased (MAD), and started neutron diffraction studies. Each of these techniques requires special crystal development. We have obtained different crystal forms, each adapted for a different study, e.g., different space groups: P21 crystals diffracting to ultra-high resolution for native data collection, P1 crystals (smaller unit cell) for neutron studies, and P212121 crystals (smaller data collection zone) for MAD studies. We have also tried different solution and protein types, e.g. SeMet derivatives for MAD studies and crystal growth in D2O for neutron studies. Each one of these crystals required a special optimisation. As a first result of this project, we will present the structure of a complex Aldose Reductase-inhibitor obtained from X-ray crystallographic data extending up to 0.62 Å and refined at 0.66 Å, the highest resolution ever recorded for an enzyme of this size. The subatomic resolution structure shows many details unavailable at lower resolution, such as H-atom positions, significant deviations from standard stereochemistry, exact determination of atomic species, bond electron density, multiple conformations and detailed solvent structure. This accuracy enables the unambiguous assigning of the orientation of His110 ring around the Cα-Cβ bond and the positioning of hydrogen atoms involved in catalysis, leading to a new reaction mechanism where both Tyr48 and His110 play a role.