Crystallography News
Sealy Center for Structural Biology and Molecular Biophysics
SCSB
X-Ray Users Group News No. 2. Spring 2010

Hotel Occupied

Crystallization trials now have a place to go and relax before their big photo session. The Rigaku Gallery 162 "Hotel" for the Minstrel tray imager holds up to 162 SBS crystallization trays at a time, in six suites. Each suite, or cassette, holds up to 27 SBS trays or 21 Linbro type plates. Currently we have five SBS suites and one Linbro suite available for your trays.

The Rigaku Gallery162 "Hotel" automatic plate loader attached to the Minstrel.

The SCSB and the department of Biochemistry and Molecular Biology worked together to provide this important addition to the Rigaku CrystalMation® Robotics system installed in August 2007, which has seen heavy use since. This technology provides SCSB members and collaborators with automation at each step, from custom screen preparation to crystallization trial imaging and analysis. The Hotel will enable systematic tracking of crystallization results, and greater integration of data and information storage and sharing.

On Target – Hit your Drops!

The *Crystaltrak*® system (CT) provides many tools for imaging your drops. The drop centering tool allows the user to specify the center of the image. This enables the user to effectively center the drop in the image, which is particularly useful for higher magnification (smaller FOV). tracking and sharing your crystallization data.

Say Cheese – Manual Photos!

The *Minstrel*® software provides many tools for imaging your drops. When you have a particularyl nice crystal that you would like to immortalize, why not use the manual [Aquire Image] feature.

Crystallization Chills

Arrval of the new Thermo Scientic 4ºC Incubator has given the researchers the chills. This Incubator replaces an identical unit which left with Dr. Bryan Sutton. Our current ability to perform crystallization trials was limited by the lack of space in the 4ºC rooms available. The incubator offers a large amount of space in a low-vibration environment more conducive to crystal growth. Trays from the incubator can be viewed using the microscope in the large 6th floor 4ºC environmental room, BSB6.624.

Missing Protein Sample - Found

The *PHOENIX*® sometimes produces trays which are missing sample in the last row or two. We have found out why this is the case. The PHOENIX is a complicated liquid handling robot specifically designed for work with 96 well crystallization trays. It is a highly adaptable system, that can handle a wide variety of sample and buffer types. Through its programming interface it is possible to optimize how it performs an experiment. Unfortunately, this also means that unless the program is suited to the particular experimental conditions, it may not perform properly. One instance of this has to do with sample viscosity.

The Nano nozzle sample handling "syringe" is a complex liquid handling device. It uses a vacuum to Aspirate sample and pressure to Dispense it. Metering is accomplished through a magnetic control valve and by adjusting the pressure or vacuum settings. The later is determined by the "**Liquid Class**". The default liquid class used for our protein samples is "**Aspirate Water**". To conserve sample we use a conservative 23uL for a 200nl drop on drop experiment. If the protein sample viscosity is a little higher than expected then less sample will be Aspirated than programmed. The effect is that the last row or two of a crystallization plate will be lacking sample, and the air blast from the nozzle may disturb the buffer droplet. Note that he

Dispense volumes of the Nano nozzle are much less sensitive to the sample viscosity and will be correct even when using more viscous samples while still specifying "Dispense Water". There are several solutions to the sample aspirate problem; aspirate using a larger requested volume, or use the correct viscosity setting to aspirate the requested volume. I have created a new Liquid Class to help correct the error in sample volume aspiration. The new Liquid Class is "**Aspirate Protein**". The Aspirate Protein class has a viscosity wich is 25% higher than the Aspirate Water class, and should correct the Aspiration errors for proteins which exhibited higher aspiration viscosity than would be expected. I do not recommend using the Aspirate PEG or Aspirate 20% Glycerol liquid classes, in most cases, as these will use more protein sample than required.

How do you know when to use the Aspirate Protein or the Aspirate Water liquid class for your sample? There is no way of knowing, ahead of time, how viscous your protein sample will appear to the Nano nozzle. Therefore I have created a simple sample viscosity test program "**010 Sample Viscosity Test - 2010-05-06**". The program will Aspirate a fixed volume (50ul) from vial A1 and dispense it all into vial A2. Measuring the volume of sample dispensed into vial A2 will let you know if the viscosity setting (Liquid Class) is correct. A simple comparison of the volumes of the two vials should let you know. For example if you put 100ul into vial A1, then after the test there should be 50ul in each vial. When finished with the test recover the used sample for use in your experiment.

If you experience missing drops in your crystallization tray then sample viscosity could be the culprit. Changing the Nano Aspirate setting to "**Aspirate Protein**" could solve this problem, if it is not due to sample pathologies such as aggregation. A simple test program "**010 Sample Viscosity Test - 2010-05-06**" can be used to evaluate the different Liquid Classes prior to setting up a crystallization tray. Thereby saving your precious sample for a successful experiment..

Don't Rain on My Crystals

The *PHOENIX*® guideplate has a great affinity for certain crystallization buffers. If you look at the underside of the plate during use you will see a meniscus formed between some of the needles and the guideplate. Some solutions will form large droplets on the tips of the needles. The solutions which form the meniscus will not be cleaned away by the water bath. To remove these drops it is necessary to pat-dry the underside of the guideplate with a Kim wipe.

Barcodes Rule!

With the arrival of the Hotel the importance of placing bar codes on every tray is now even greater. We have always had a policy of barcoding every tray setup on the *PHOENIX*®. We strongly recommend the same for your Linbro trays too. This way the experimental details are recorded in the CT database, and the Hotel can automatically image your tray.

Semper Paratus, Semper Vigilans, Semper Video

The Rigaku Hotel and Minstrel stand ever ready, ever waiting, and always looking at your trays. So make use of the Hotel to store your plates. It's also the ideal isothermal chamber for storage of crystallization trays in the 17° C room.

XUG Meeting Briefs

An expanded X-Ray Users Group (XUG) meeting resumed this Fall with a talk highlighting the expended field of topics discussed. Dr. Mitul Saha, from Dr. Marc Morais' Group, presented his talk on EM model fitting "MOTIF-EM: an Automated Computational Tool for Identifying Conserved Domains in CryoEM Structures". This month's presenter, Dr. Cecile Bussetta, from Dr. Kay Choi's group, presented a multifaceted presentation of her ongoing work "Dengue virus NS5 protein: structure determination using X-ray crystallography and small-angle X-ray scattering". XUG meetings are held as a small group meeting with

informal presentations where we discuss all aspects of crystallography, scattering, and Cyro Electron Microscopy from protein expression to structure refinement and analysis. We have also met to participate in webinars given by prominent researchers such as Ian Wilson, Wladek Minor, and James Pflugraph. We recently had a presentation by Matt Benning, of Bruker-AXS, highlighting in-house structure solution using SAD. In May we will be hosting Michael Rossman, and a BioSAXS webinar. XUG meetings are usually held on the 4th Wednesday of each month, at noon, in MRB 6.102. To get on the mailing list contact Dr. Mark A. White mawhite@utmb.edu. Upcoming meetings are listed on the SCSB X-Ray Diffractometer Scheduling calendar (http://xray.utmb.edu/xcal).

Safety Rules!

Safety is a very important concern. In this regard we have rules and procedures which are designed to minimize the danger from x-ray radiation. Please download and read the Radiation Safety Test form from our web-site and review the safety information on our website. Look for more information about the instruments and safety in the **Protocols** section under **Info**.

CAMD - PX1: Beam Time

E-mail Henry Bellamy to request time available (gcpcc_access@xray.utmb.edu).

Beam time begins at noon of 1st day.

Radiation Safety - PX1 Ring Current Directions, Hotels

Contacts: UTMB – CAMD - **(PX1 225.578.7137)**

Policy Matters

SCSB Robotics Instrumentation Use Policy

Now that the Hotel is here and working it is an ideal time to set Center policy on the use of the instruments and to require the adoption of good data management protocols. This is the Policy, developed by the SCSB Crystallography Faculty, regarding use of the Crystallization robots.

In keeping with University policy regarding data recording, archiving, storage, and access the SCSB faculty have adopted the following policies.

1) All Instrument use must be recorded. All use of the Center Instrumentation must be recorded in the **Scheduling Calendar** and in the paper logbooks. This includes the Rigaku CrystalMation Robotics, NMR Spectrometers, and X-Ray diffraction systems and other instruments. Details of the experiment should be recorded in the appropriate place. For billing, the project PI and the actual resources used, such as the number of trays used (including failed experiments), days of diffractometer use, etc., must be recorded where indicated. Any problems with the robotics should be noted and reported to the manager immediately.

2) All crystallization trays must be labeled with a barcode. All crystallization trays created in the SCSB facilities must be labeled with a barcode generated using the *Crystaltrak* (CT) system. The twelve character plate name printed on the barcode should include the PI's initials, the sample name and the screen used. We suggest the following format: **PiiSampleScr : ie. McmPhi29aWz3, KycNpro09JCS, VjhAK8V08Idx, FoxYcie9a1SS**. This name is the first twelve characters of the plate name entered in CT, which may be longer than twelve characters, but only the first twelve are printed on the barcode label. Note that the barcode label automatically includes the date of creation and the barcode ID, so these do not need to be in the plate name.

3) All trays must be imaged using the Minstrel. When it is consistent with good experimental design, all trays which are part of a Center funded project or use Center resources, must be imaged using the Minstrel,

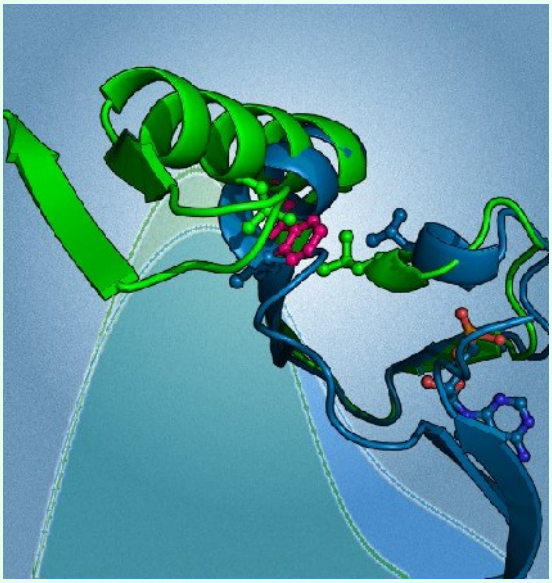
and the results recorded in *Crystaltrak.* Trays maintained at a constant temperature other than 17° C are not required to be imaged in the Minstrel. The results of all crystallization trials must be recorded in *Crystaltrak* (CT). Records of crystallization results can include "**tag**"ing or **scoring** potential hits, or simply adding a comment in the "**Plate Notes**" box denoting the presence or absence of crystals. Individual PIs may require additional documentation. We highly recommend that all researchers create CT barcodes for their 24 well Linbro-style plates also, and track their progress in CT.

4) All data must be archived. **X-Ray diffraction data** must be **archived** onto a **DVD**, or other external media for the users own use. This data must not be erased by the user from the main data storage directories (c:\frames or /DIP1/data).

Scheduling Policy Links: X-Ray Diffractometer Scheduling Policy
–
Crystallization Robotics Scheduling Policy

Molecule of the Month

Epac2



Superposition of the cAMP activated switch region in Epac2. The green and blue cartoons show the apo- and cAMP bound conformations respectively. Key residues involved in cAMP activated switching are also shown, particularly F435 (colored magenta) which regulates the switch. The SAXS P(r) curves, also colored green and blue, are shown in the background. The increase in the Rg of the cAMP activated molecule is clearly noticeable in the P(r) curves.

Tsalkova T, Blumenthal DK, Mei FC, White MA, **Cheng X**. Mechanism of EPAC activation: Structural and functional analyses of EPAC2 hinge mutants with constitutive and reduced activities. *J Biol Chem.* **284;** 23644 – 23651, Aug. 28, 2009 PMID: 19553663