

Crystallization Trials Moved into Hotel

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 Gallery 162 "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.

Press-n-Go

Crystallization tray imaging was never easier. Now with the Hotel here and working it is as simple as pressing a button. When a user wishes to examine a tray by eye, he/she can simply select the tray barcode ID in the Minstrel's Inventory window (lower left) and (UNLOAD) it with a single click of the mouse. Returning the tray, or loading a new tray is even simpler. Just press the [LOAD/UNLOAD] button on the Hotel front



The Hotel's tray carriage door and LOAD/UNLOAD Button (right)

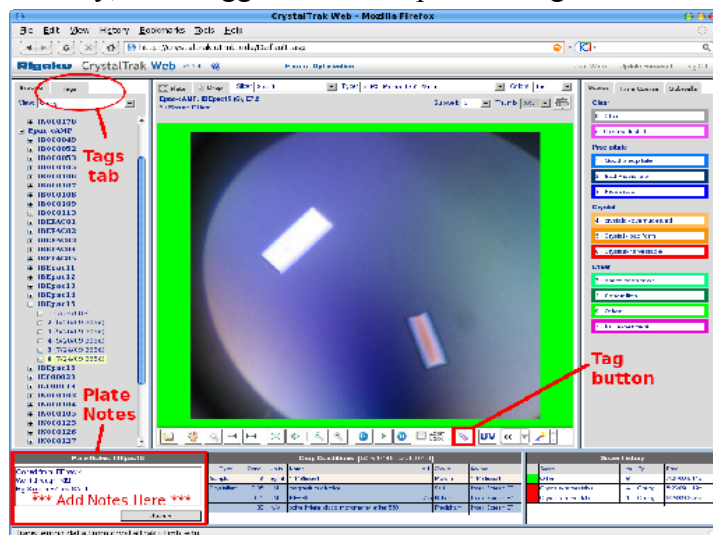
(shown at right), and the carriage door opens. You place your tray on the carriage, and then press the button once more. Now you can go and continue with your day while the Minstrel performs its magic. The Minstrel software will automatically schedule the tray for imaging according to the settings you entered in *Crystaltrak*® when creating the plate barcode. The default schedule images each tray once per week. If you want to image your tray immediately, just select your tray in Minstrel software's inventory window (lower left) and then click on the [Inspect] button.



The button

Tag - You're it!

The *Crystaltrak*® system (CT) provides many tools for tracking and sharing your crystallization data. One of these tools is the **Tags** tab in the CT Results and CT-Web tray views. The act of "Tag"ing a well places that image in a list of images which all share the same tag. Users can create as many tag lists as they desire. Initially, it is suggested that a private Tag list be cre-



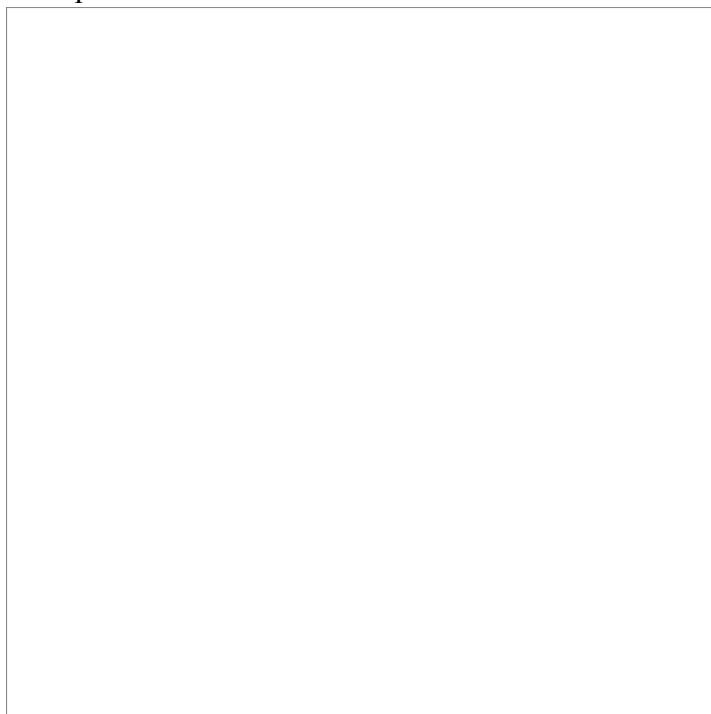
ated for each project, such as "AK-Crystals-09". Then new results can be quickly added to the list by project members and the images easily found by other members of the group. Do not forget to "score" the image and to add notes in the **Plate Notes** window. Look for the **Tags** tab at the top of the left frame, above the list

of tray IDs. Images can be "Tagged" either by clicking on the Tab icon (right), beneath the image, or from the right-mouse menu.



10° C Cold Room goes Hot

Crystallization trials sometimes need to be done at just the right temperature. Trials are commonly run at 4° C, 10° C, 15° C, and room temperature (20°-25° C). We have been lacking the ability to run trials at 10° C, but no longer. By converting the environmental room in BSB 6.627 (#105) from 20° C to 10° C we add an important capability while still having access to the adjacent 17° C room. The room was turned down to 10° C on September 22nd.



Label it

We have started to assign shelf space in the common areas used for crystallization. Space has been assigned to active groups, one shelf per project/researcher (see diagram above). This will help groups to manage their trays better and aid in the clean-up of old trays. Each assigned shelf is clearly labeled. Additional labels are on the first shelf on the right in the 17° C room.

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 Meetings Resume

An expanded X-Ray Users Group (XUG) meeting resumed this Fall with a talk highlighting the expanded 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 Cryo Electron Microscopy from protein expression to structure refinement and analysis. 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 web-site. Look for more information about the instruments and safety in the **Protocols** section under **Info**.

CAMD - PX1: Beam Time

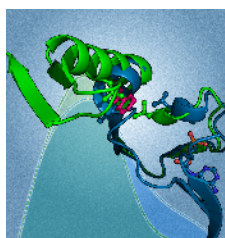
E-mail **Henry Bellamy** to request time available (gcpcc_access@xray.utmb.edu).

Beam time begins at noon of 1st day.

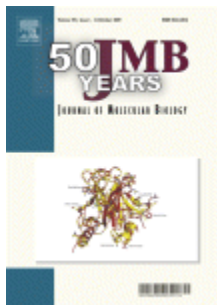
[Radiation Safety](#) - [PX1 Ring Current](#) [Directions](#), [Hotels](#)

Contacts: [UTMB](#) – [CAMD](#) - (PX1 225.578.7137)

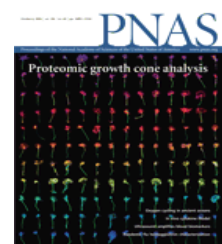
SCSB Publications (See the [full list](#))



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 [Epub-2009 Jun 24]. PMID: 19553663



Szymanski MR, Fiebach AR, Tratschin JD, Gut M, Ramanujam VM, Gottipati K, Patel P, Ye M, Ruggli N, **Choi KH**. Zinc-Binding in Pestivirus N(pro) is Required for Interferon Regulatory Factor 3 Interaction and Degradation. *J Mol Biol.* Aug. 14, 2009 [Epub 2009 Jun 18.] PMID: 19540847



Schrank TP, Bolen DW, **Hilser VJ**. Rational modulation of conformational fluctuations in adenylate kinase reveals a local unfolding mechanism for allostery and functional adaptation in proteins. *Proc Natl Acad Sci U S A.* 2009 Oct 6;106(40):16984-9. Epub 2009 Sep 21. PMID: 19805185

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.

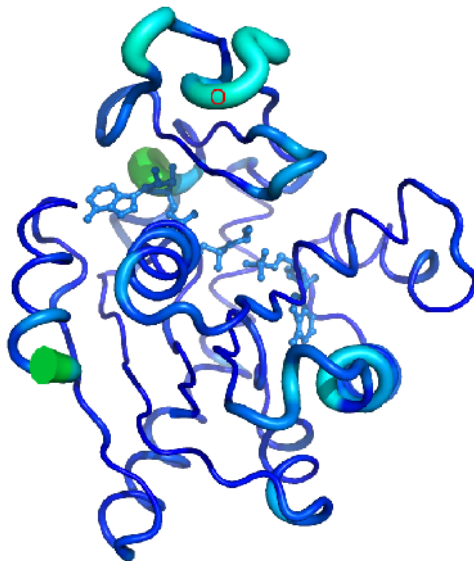

3) All trays must be imaged using the Minstrel. When it is consistent with good experimental design, all trays which are part of a Center funded project or use Center resources, must be imaged using the Minstrel, and the results recorded in *Crystaltrak*. Trays maintained at a constant temperature other 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](#)

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[Crystallization Robotics](#)
[Scheduling Policy](#)

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|  | <p style="text-align: center; font-weight: bold;">Molecule of the Month</p> <p style="text-align: center; font-weight: bold; margin-top: 20px;">Adenylate Kinase</p> <p style="margin-top: 20px;">RMSD worm figure of Adenylate Kinase G148V mutant drawn using the PMB pmb_Colour_by_Rmsd script and PYMOL. The site of mutation is circled. The maximum Cα r.m.s.d. to the WT is 0.63 Å. The maximal positional deviations are in two loops of the flexible lid domain containing the site of mutation.</p> <div style="text-align: right; margin-top: 20px;">  </div> |
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