Crystallography News

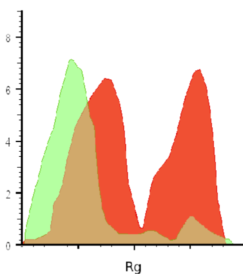
Sealy Center for Structural Biology and Molecular Biophysics

SCSB The Sealy Center for Structural Biology and Molecular Biophysics

X-Ray Users Group News **No. 3. Fall 2010**

Complete your Ensemble with SAXS

The new fashion in macromolecular structure determination this Fall is Small Angle X-ray Scattering (SAXS). While crystallization results in a static picture of a single conformation of your protein. In solution your molecule may adapt one, two, or several conformations. Cryo-EM offers one method of imaging samples in solution, Small Angle X-ray Scattering (SAXS) provides another complementary method. In the last several years analysis tools for SAXS data have grown to include tools for querying ensembles of structures to find those that best match the solution scattering profile. Two programs that do this are: MES (Hammel) and EOM (Svergun). These tools are to be found amongst the SAXS toolkit available in the SCSB Crystallographic & EM Computational Lab.

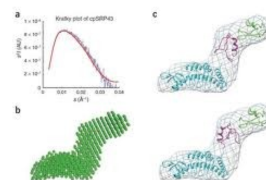


The SAXS Ensemble Profile of Epac2 shifts with cAMP binding from the green (apo) to the red (holo) ensemble.

The Big 'GUN Comes to Town

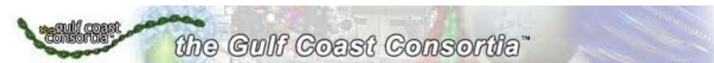
The author of the famous ATSAS suite of SAXS and BioSAXS data processing and analysis tools , Dimitri Svergun, gave a lecture to a room full of interested researchers, on December 7th. His talk, entitled *"Small Angle X-ray Scattering from Macromolecular Solutions and Nanoparticles"* reviewed the latest developments in BioSAXS analysis. His talk was interspersed with delightfully humorous video illustrations of each key point, making the hour fly past quickly. Although his software descriptions were ATSAS based, the key points were generic to the SAXS technique and most valuable to the SAXS newbie. The discussion touched upon the benefits and limitations of the SAXS technique. His opinion that much SAXS work can be done with home systems as easily as at synchrotron beamlines was encouraging to many in attendance, especially considering his role as group leader of the EMBL/DESY X33 SAXS beamline group.

SAXS is a low resolution technique, in which the data rarely exceeds 10-15Å resolution. Unlike CryoEM SAXS does not produce images and molecular envelopes, but rather a scattering profile from the isotopically averaged protein in solution. Therefore, the structure solution may not be unique, and over-fitting is to be avoided. However, SAXS can provide much useful information about protein conformational changes, complexes, and intrinsically disordered regions of proteins. Even protein:DNA or RNA complexes can be examined using contrast variation techniques. All this can be accomplished with limited sample preparation; SAXS requires only your protein (complex) in solution.



Solution Structure Workout: Swim the SAXS river

Interested parties are invited to attend a BioSAXS Workshop in the Woodlands on January 25th, 2011. The workshop, co-sponsored by Rigaku and the GCC SAXNS Group, will feature a series of morning lectures, followed, in the afternoon, by a practical workshop on the use of the ATSAS tools.



Join the Conquering SAXNS

You are invited to joint the GCC Small Angle X-ray & Neutron Scattering interest group: The SAXNS. This is a group of researchers currently using or intending to apply SAXS or SANS to conquer difficult structural biology problems. The group is organized by Xiaodong Cheng, and the SCSB at UTMB, but includes members from most GCC in-

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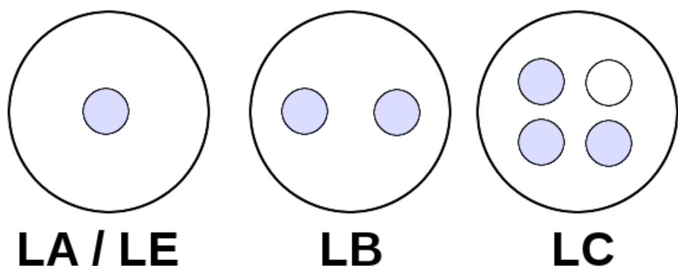
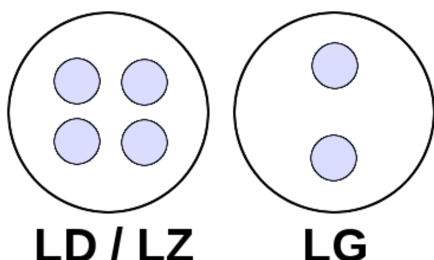
stitutions. For more information see the SAXNS web site http://xray.utmb.edu/SAXNS.

Automated Manual Tray Imaging

The *Minstrel*® software includes definitions for those 24 well hanging drop "Linbro" plates that you set up by hand. Its just a matter of choosing the right code to match your setup. For instance, the typical single hanging drop tray should use the **LA** code. For double drops in line with the long (6 well-axis) row of the plate use **LB**. Note that the three-drop format (**LC**) is not an equilateral triangle of drops, but as-

LINBRO





sumes a square layout missing the upper right corner drop of the four-drop layout **LD**. If you setup your two drops along the short axis of the tray then use the **LG** code.

Round & Round They Go

The Centers new Eppindorf 8504 DWB Centrifuge is configured to spin down the 96-well crystal screen Deep Well Blocks (DWB). This helps to remove any precipitant matter from the solution before using it for crystallization trials. Don't forget that we have a Spectrafuge 24D centrifuge that you can use for your 1mL sample vials to remove any precipitant from the sample before setting up your crystallization trays.

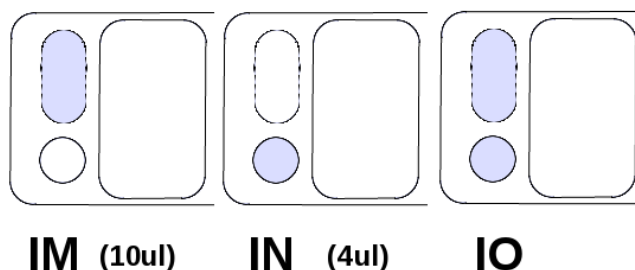
Cryschem Sits for Portrait

If you use the moulded **CrysChem** sitting drop trays then use the **V0** "*Victor-Zero*" plate code for imaging these plates in the *Minstrel*®.

Remember Your ARI Codes

The Art Robbins Intelliplate 2-well SBS format crystallization trays are identified by the following CT codes. The most commonly used format is the **IN** code for the small 4µl well.

ARI Intelliplate



Take your trays for a spin

The Eppindorf 8504DWB Centrifuge can also spin your SBS format crystallization trays. This produces drops of uniform size, and merges droplets within the well. Try it, you'll like it! & so will your trays.

Yearly Radiation Safety Test - Deadline approaches!

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. As part of this a yearly review of the Safety Policy of the Center is required. Please download and complete 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**. New login access information will be sent to all authorized users in the new year. The deadline for submitting this year's test is December 31, 2010.

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#@!%& Keep It Clean!

To avoid foul mouths and fouled guide-plates cleanliness is vital. The Phoenix guide-plate, that aligns the 96 syringes, recently became fouled with died buffer solutions. The reason for this was not obvious. The user had followed all the protocols and had not seen any obvious signs of guide-plate fouling: missing drops, overfilled drops or over-spray. So with the help of our friends at UH and Rigaku (Kris Tesh, and Jeff Myers) we have found a possible cause of this serious problem: The Wizard screen kits. The Wizard screen kits come in DWBs that are sealed by a silicone gasket. We all know that this gasket requires special care, and significant force, to seal properly, but is can also create problems when opened improperly. The partial vacuum created by the seal can pull buffer up onto the top of the DWB. These drops can then stick to the syringes or touch the guide-plate directly. If you think that this may have happened then it is vital to hand wash the guide-plate with water. Air-dry the plate, with compressed air (there is a small hose next to the Phoenix) to remove any remaining water. Remember, cleanliness is ... defined as being ”Habitually and carefully neat and clean.”

I Need A Microscope Here!

The SCSB X-ray Crystallography Lab has a microscope where you need it. At room temperature we have two microscopes, a high-power Leica MZ12.5/Spot-CCD and an Olympus SZ6. Another Olympus SZ6 is housed in the 4ºC room (BSB 6.624). In the 10C ecrystallization room we have an Olympus SHZ10/DP-20 system, and in the 17ºC room is the Rigaku *Minstrel*®. The Olympus SZ6 microscope next to the X-ray diffractometers is primarily for conducting experiments on these instruments. Users must yield access to anyone collecting data on the instruments.

Crystallization Chills

Arrival of the new Thermo Scientic 4ºC Incubator has given crystallographers 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.

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)**

The Pellet

SAXS Kratky plot and molecular envelopes from: Peera Jaru-Ampornpan, Kuang Shen, Vinh Q Lam, Mona Ali, Sebastian Doniach, Tony Z Jia & Shu-ou Shan. ATP-independent reversal of a membrane protein aggregate by a chloroplast SRP subunit, *Nature Structural & Molecular Biology* Volume: **17**, 696–702 (2010).

Cleanliness quote: www.thefreedictionary.com/Cleanliness

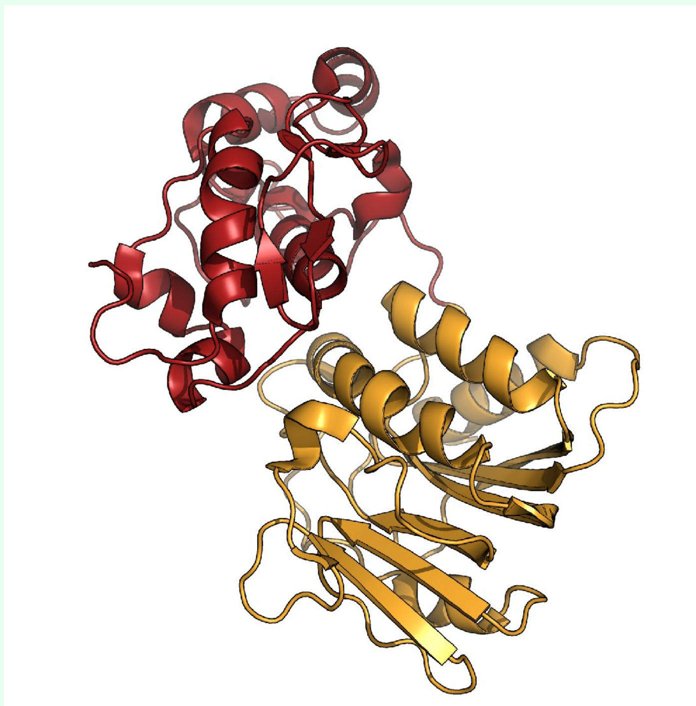
Saxon Cartoon from: *www.earlybritishkingdoms.com*
Picture of the helmet from the ship-burial at Sutton Hoo is from: *www.britishmuseum.org*

The **SAXNS** are named after the scattering technique used for probing the low-resolution structure of macromolecules in solution. The Saxons were named, by the Romans, after the iron sword or Sax which they used. Please do not confuse the two.

- Thank you, *Haroldus Rex*

Molecule of the Month

VEE Nspro2 Viral Protease



Structure of the two minimally required domains of VEE nsp2.

The alphavirus nsP2 protease is essential for correct processing of the alphavirus nonstructural polyprotein (nsP1234) and replication of the viral genome. We have combined molecular dynamics simulations with our structural studies to reveal features of the nsP2 protease catalytic site and S1′–S4 subsites that regulate the specificity of the protease. The catalytic mechanism of the nsP2 protease appears similar to the papain-like cysteine proteases, with the conserved catalytic dyad forming a thiolate-imidazolium ion pair in the nsP2-activated state. Substrate binding likely stabilizes this ion pair. Analysis of bimolecular complexes of Venezuelan equine encephalitis virus (VEEV) nsP2 protease with each of the nsP1234 cleavage sites identified protease residues His510, Ser511, His546 and Lys706 as critical for cleavage site recognition. Homology modelling and molecular dynamics simulations of diverse alphaviruses and their cognate cleavage site sequences revealed general features of substrate recognition that operate across alphavirus strains as well as strain specific covariance between binding site and cleavage site residues. For instance, compensatory changes occurred in the P3 and S3 subsite residues to maintain energetically favourable complementary binding surfaces. These results help explain how alphavirus nsP2 proteases recognize different cleavage sites within the nonstructural polyprotein and discriminate between closely related cleavage targets.

Russo AT, Malmstrom RD, White MA, Watowich SJ. Structural basis for substrate specificity of alphavirus nsP2 proteases. J Mol Graph Model. 2010 Aug 24;29(1):46-53. Epub 2010 Apr 24.PMID: 20483643.