

ISB3D X-ray Crystallography Facility

A. Overview

The X-ray Crystallography laboratory, which is part of a structural biology core facility, is a user-friendly facility located at the Institute for Structural Biology, Drug Discovery and Development (ISB3D), Virginia Commonwealth University (VCU). The facility provides investigators equipment and resources for crystallographic analysis of macromolecules and/or small molecules.

The facility has operated various generations of X-ray diffraction equipments since 1988. The first was Rigaku AFC-5 four-circle diffractometer/RU-200 generator in 1988. The AFC-5 was replaced with a Rigaku R-axis II in 1997. In 2004, The R-axis II/RU-200 was replaced with a Rigaku R-axis IV++ Image Plate/Micromax 7 Generator using an NIH National Center for Research Resources (NCR) Shared Instrumentation grant. In 2017 the X-ray equipment was replaced with a new state-of-the-art Rigaku MicroMax-007HF X-ray Generator with VariMax-HF Arc Optics/Hybrid Photon Counter, Eiger R 4M Detector and AFC11 Goniometer using an NIH Shared Instrumentation grant supplemented by Virginia General Assembly Higher Education Equipment Trust Fund (HEETF).

The crystallography facility offers and operates as a full service core by performing crystallization, X-ray diffraction data collection processing, phasing, crystallographic refinement, model building, and visualization. The structural data obtained by the core will provide scientists with a wealth of information including but not limited to 3D-structure, biological functions, structure-based drug design, ligand or DNA binding to protein, mutational effect of target macromolecules, or absolute stereochemistry of chiral compounds.

The facility is complemented by a variety of training mechanisms and services to enhance access and user capability. In addition to providing service to the VCU community, we also offer crystallography services to outside organizations. Please contact us for your crystallographic and other structural biology service needs and/or potential collaborations.

B. Available Services

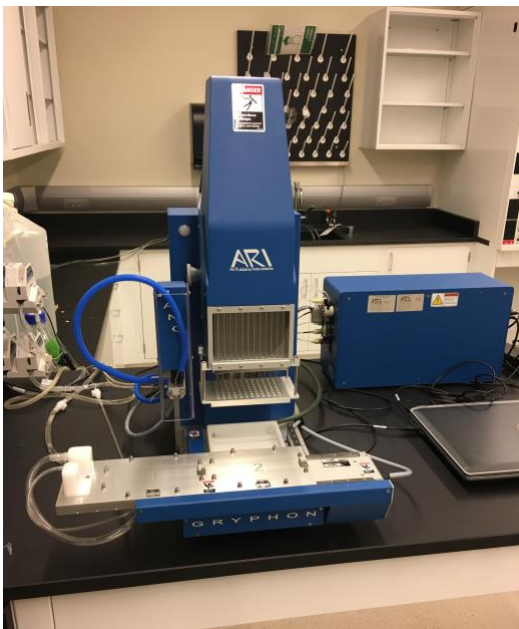
- Feasibility determination/studies
- Crystallization of small molecules and macromolecules
- Characterization of crystals
- Diffraction data collection at ambient temperature and 100K
- Data analysis and processing
- Structure determination and analysis

Note: We expect all users to be appropriately trained to utilize the X-ray instrument and other equipments, particularly taking into account the radiation safety measures. If you need training please contact Dr. Faik Musayev (fmoussae@vcu.edu)

C. Equipment and Resources

- Gryphon Crystallization Robot, which can set up nano liter drops in minutes

- Alchemist Liquid Handling System
 - Minstrel/Gallery Imaging
 - Crystallization Incubators set at different temperatures for crystallization experiments
 - CrysCam Digital Microscope for monitoring crystal growth and manipulation
 - Olympus SZ51, Nikon SMZ-2T Microscopes for inspecting crystals
 - X-ray Instrument and accessories
- AFC11 Goniometer
 - Hybrid Photon Counter
 - Eiger R 4M Detector
 - MicroMax-007HF X-ray Generator
 - VariMax-HF Arc Optics
 - Oxford Cobra Cryo-system for data collection at 100K
 - NitroFlowLab Nitrogen Generator
- Computational facilities for diffraction data collection, processing and structure determination
 - Crystallographic and modeling software packages



Crystal Gryphon Crystallization Robot



Minstrel/Gallery Imaging



MicrMax-007HF X-ray Generator



Oxford Cobra Cryo-system

D. User Fee

Description	Rate	Quantity
Crystallization Robots		
Tray	\$10.00 (VCU), \$15.50 (External)	Per tray
Crystal Imaging (Formulatrix)		
Imaging fees	\$2.00 (VCU), \$3.10 (External)	Per tray
Diffraction Data Collection		
Macromolecules or Small Molecules	\$40.00 (VCU), \$62.00 (External)	Per hour (maximum charge for a day \$120.00 (VCU), \$186.00 (External))

E. X-ray Time Request (login or register to have login access)

- To reserve the instrument, please go to the link <https://rams.research.vcu.edu>.
- We expect all users to be appropriately trained to operate and utilize the X-ray instrument and other equipments, particularly taking into account the radiation safety measures. If you need training and/or help to operate the instrument, please contact Dr. Faik Musayev (fmoussae@vcu.edu)
- **Note:** First time users require radiation safety training prior to operating the X-ray instrument. The Radiation Safety online training is offered through Blackboard. To enroll in this course, login to Blackboard and search for course "Radiation Safety Training for Research Laboratory Employees". Once you have enrolled, follow the instructions on the Course Documents page. The radiation safety-training manual is also found [here](#).

F. Publications (selected)

Chiang, M.-J., Musayev, F. N., Kosikova, M., Lin, Z., Gao, Y., Mosier, P. D.; Althufairi, B., Ye, Z., Zhou, Q., Desai, U. R., Safo, M. K., Xie, H. Maintaining pH-dependent conformational flexibility of M1 is critical for efficient influenza A virus replication. *Emerging Microbes & Infections*, 2017 Dec 6; 6(12): e108. doi: 10.1038/emi.2017.96.

Xu, G. G., Pagare, P. P., Ghatge, M. S., Safo, R. P., Gazi, A., Chen, Q., David, T., Alabbas, A. B., Musayev, F. N., Venitz, J., Zhang, Y., Safo, M. K., Abdulmalik, O. Design, synthesis, and biological evaluation of ester and ether derivatives of antisickling agent 5-HMF for the treatment of sickle cell disease. *Mol Pharm*. 2017, 14(10), 3499-3511.

Ghatge, M. S., Ahmed, M. H., Omar, A. S., Pagare, P. P., Rosef, S., Kellogg, G. E., Abdulmalik, O., Safo, M. K. Crystal structure of carbonmonoxy sickle hemoglobin R-state conformation. *J. Struc. Biol* 2016, 194(3), 446-50.

Yang J, Nune M, Zong Y, Zhou L, Liu Q. Clos and Allosteric Opening of the Polypeptide-Binding Site in a Human Hsp70 Chaperone BIP. *Structure* 2015, 23 (12), 2191-203.

Remesh SG, Santosh V, Escalante CR. Structural Studies of IRF4 Reveal a Flexible Autoinhibitory Region and a Compact Linker Domain. *Journal of Biological Chemistry* 2015, 290 (46), 27779-90.

Musayev, F. N., Zarate-Perez, F., Bishop, C., Burgner, J. W 2nd, Escalante, C. R. Structural Insights into the Assembly of the Adeno-associated Virus Type 2 Rep68 Protein on the Integration Site AAVS1. *Journal of Biological Chemistry* 2015, 290, 27487-27499.

Safo, M. K., Musayev, F. N., Mosier, P. D., Zhou, Q., Xie, H., Desai, U. R. Crystal structures of influenza A virus matrix protein M1: Variations on a theme. *PLoS One* 2014, 9, e109510.

Svintradze, D. V., Peterson, D. L., Collazo-Santiago, E. A., Lewis, J. P., Wright, H. T. Structures of the Porphyromonas gingivalis OxyR regulatory domain explain differences in expression of the OxyR regulation in Escherichia coli and P. gingivalis. *Acta Crystallogr D Biol Crystallogr*. 2013, 69 (Pt 10), 2091-103.

Boundy, S., Safo, M.K., Wang, L., Musayev, F. N., O'Farrell, H. C., Rife, J. P., Archer, G. L. Characterization of the Staphylococcus aureus rRNA methyltransferase encoded by orfX, the gene containing the staphylococcal chromosome Cassette mec (SCCmec) insertion site. *Journal of Biological Chemistry* 2013, 288 (1), 132-40.

Xu, X., Marni, F., Wu, S., Su, Z., Musayev, F., Shrestha, S., Xie, C., Gao, W., Liu, Q., Zhou, L. Local and global interpretations of a disease-causing mutation near the ligand entry path in hyperpolarization-activated cAMP-gated channel. *Structure* 2012, 20(12), 2116-23.

Yi, J., Thomas, L. M., Musayev, F. N., Safo, M. K., Richter-Addo, G. B. Crystallographic trapping of heme loss intermediates during the nitrite-induced degradation of human hemoglobin. *Biochemistry* 2011, 50(39), 8323-32.

Musayev, F. N., Di Salvo, M. L., Saavedra, M. A., Contestabile, R., Ghatge, M. S., Haynes, A., Schirch, V., Safo, M. K. Molecular basis of reduced pyridoxine 5'-phosphate oxidase catalytic activity in neonatal epileptic encephalopathy disorder. *Journal of Biological Chemistry* 2009, 284, 30949-30956.

Sachdeva, S., Musayev, F. N., Alhamadsheh, M. M., Scarsdale, N., Wright, H. T., Reynolds, K. A. Separate Entrance and Exit Portals for Ligand Traffic in Mycobacterium tuberculosis FabH. *Chemistry & Biology* 2008, 15, 402-412.

Safo, M. K., Musayev, F.N., di Salvo, M., Hunt, S., Schirch, V. Crystal Structure of Pyridoxal Kinase from the *Escherichia coli* PdxK gene: Implications for the Classification of Pyridoxal Kinases. *J. Bacteriol.* 2006, 4542-4552.

Safo, M. K., Ko, T.-P., Musayev, F. N., Zhao, Q., Robinson, H., Scarsdale, N., Wang, A. H., Archer, G. L. Crystal structure of Blal repressor from *Staphylococcus aureus* and its complex with DNA: insight into transcriptional regulation mechanisms of the *bla* and *mec* operons. *Journal of Bacteriology* 2005, 187, 1833-1844.

G. Acknowledgement/Instrument Funding Citation

- All research articles that use diffraction data from the crystallography facility should cite the NIH Shared Instrumentation Grant S10-OD021756 for acknowledgement.

H. Other Structural Biology Related Services at ISB3D:

- Protein Production,
- Nuclear Magnetic Resonance (NMR),
- Molecular Modeling
- Biophysical Analysis/High Throughput Screening (HTS)

I. Personnel/Contact Information

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