Science and Capabilities on IMAGINE at the HFIR

Flora Meilleur

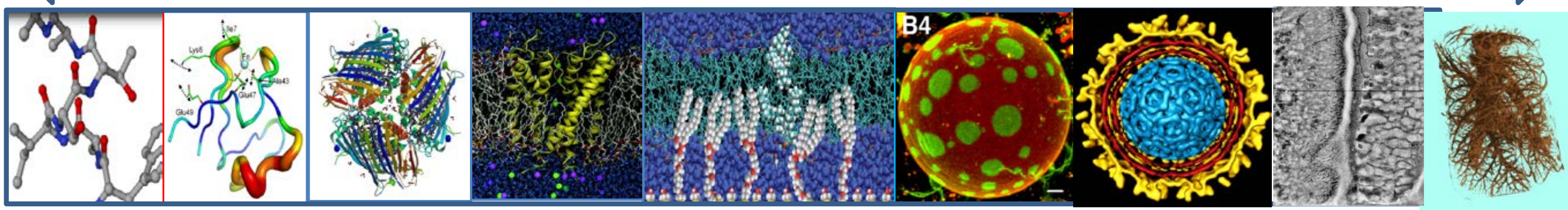
North Carolina State University, Oak Ridge National Laboratory

meilleurf@ornl.gov

Neutrons in Structural Biology: Atoms to cells

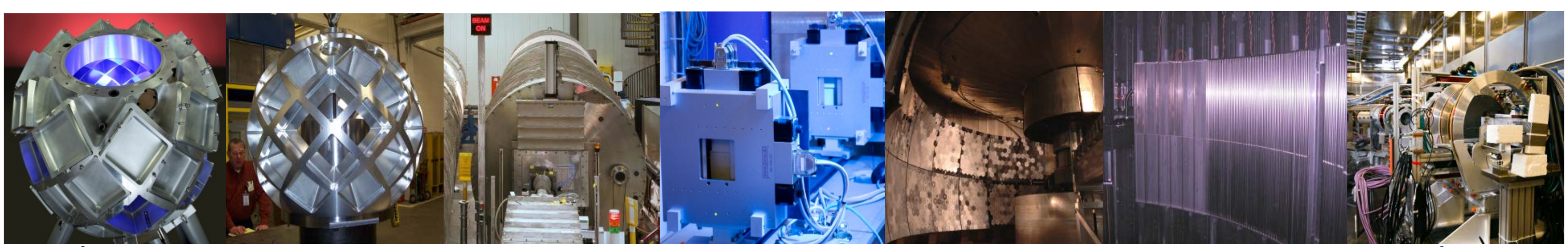
Neutrons are ideal for studying the length and time scales intrinsic to biological systems.

Atomic Femtosecond Nano Nanosecond meso Minutes cm



Sensitivity to hydrogen, the ability to deuterate, lack of radiation damage, the ability to exchange energies and high penetration provide neutron technologies with unique capabilities for biology.

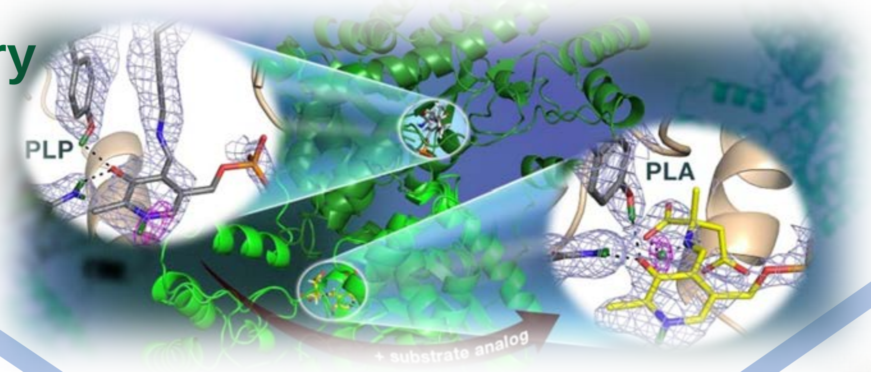
A variety of neutron techniques, including small-angle scattering, reflectometry, fiber diffraction, crystallography, imaging and inelastic scattering are available for studying biology



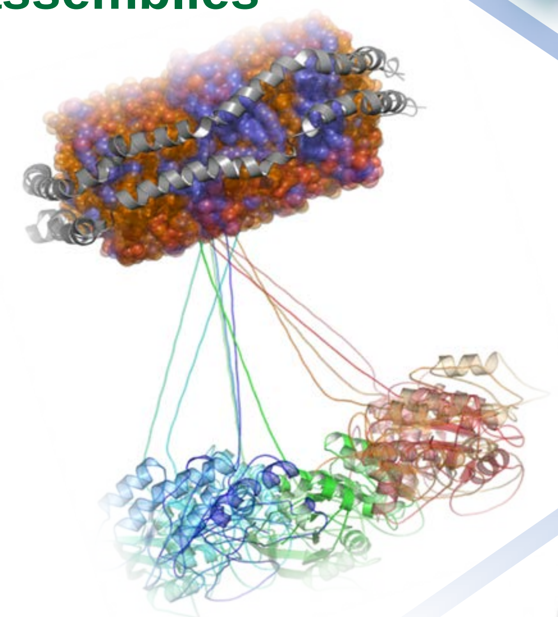
TOPAZ MaNDi IMAGINE BASIS LIQUIDS REFLECTOMETER CNCS BioSANS EQ-SANS GP-SANS NSE IMAGING

Neutrons… an ideal probe for studying complex biological systems

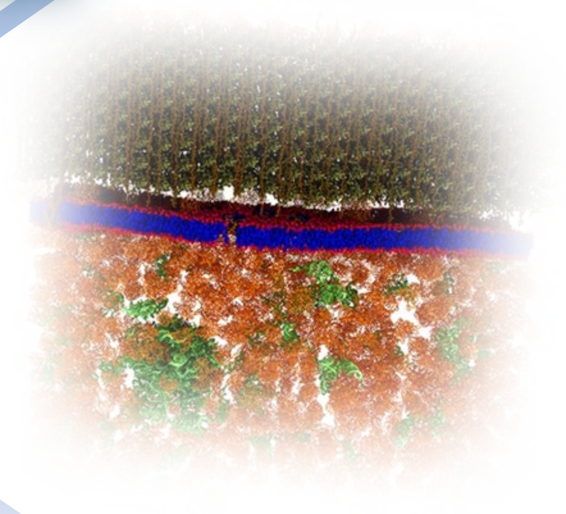
Protein chemistry



Complexes/ assemblies

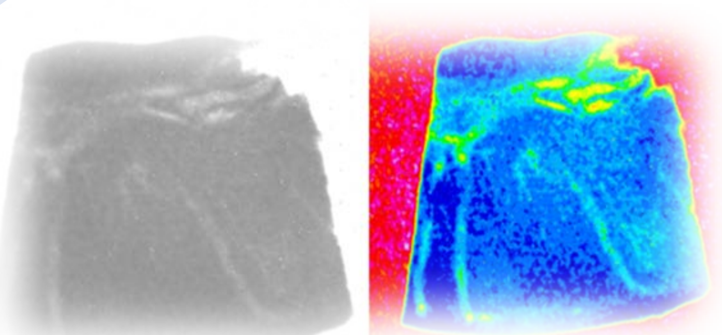


Live cell studies

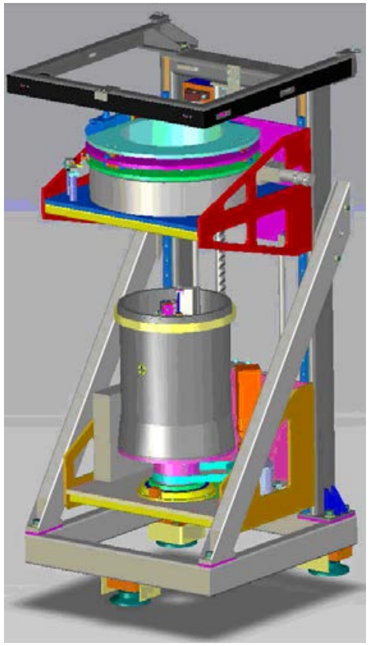


- ***Exquisitely sensitive to H/D***
- ***targeted isotope contrast***
- ***Penetrating /non-destructive***

Direct imaging



IMAGINE – A supra- and macromolecular *tunable quasi-Laue* neutron *image plate* diffractometer at HFIR



www.maatel.com

November 2007

ORNL Neutron Scattering Science Advisory Committee endorsed IMAGINE project and HFIR allocated beam line CG4

July 2009

NSF MRI award

May 2013

IMAGINE entered the user program

2013 Structural biology

2015 Dynamic Nuclear Polarization

2016 Magnetism, Phase transition

2018 High Pressure

Quasi-Laue geometry for structural biology

- X-ray macromolecular crystallography: monochromatic (X-ray beam intense)

- Neutron macromolecular crystallography: neutron beams are weak, maximize the flux of neutrons (Make every neutrons counts…): polychromatic geometry
  - Laue: density of diffraction pattern may prevent deconvolution of overlapped reflection and integration at high resolution
  - Quasi-Laue: diffraction pattern less dense

Detector

- ... Count every neutrons: Large, “wrap-around” detector:
  - $2d \sin \theta = n \lambda$
  - Because the wavelengths used are long compare to X-ray wavelengths, Bragg spots are observed at high angle
  - Example
    - » With X-ray 1 Å, 2 Å resolution diffraction spots are observed at $\theta \sim 14.5°$
    - » With neutron at 3 Å; 2 Å resolution diffraction spots are observed at $\theta \sim 48.6°$

IMAGINE beam line at HIFR

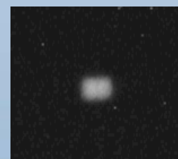
HFIR cold guide hall

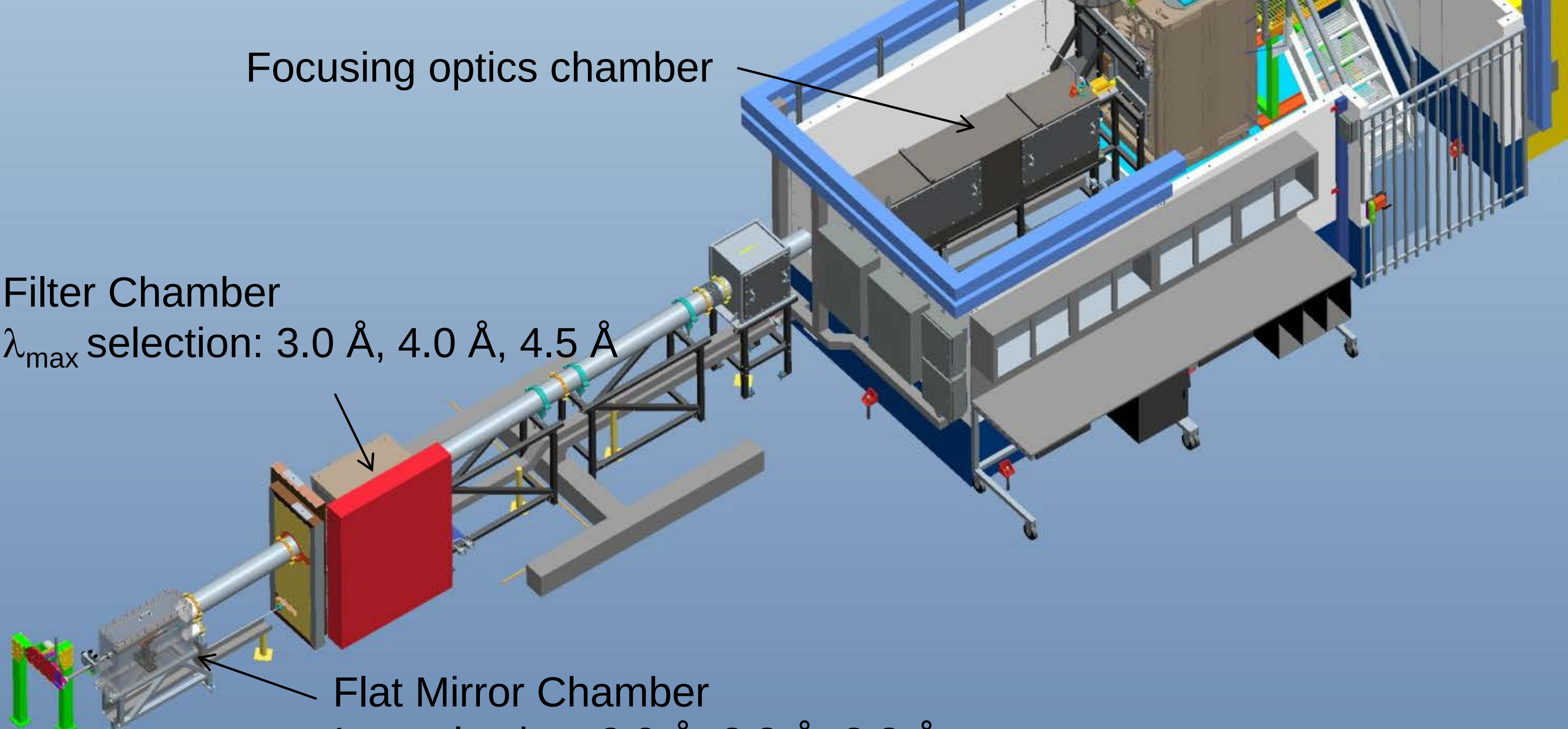
CG4 beam line end position

Neutron spectrum transmitted by CG-4: 2 Å – ~10 Å

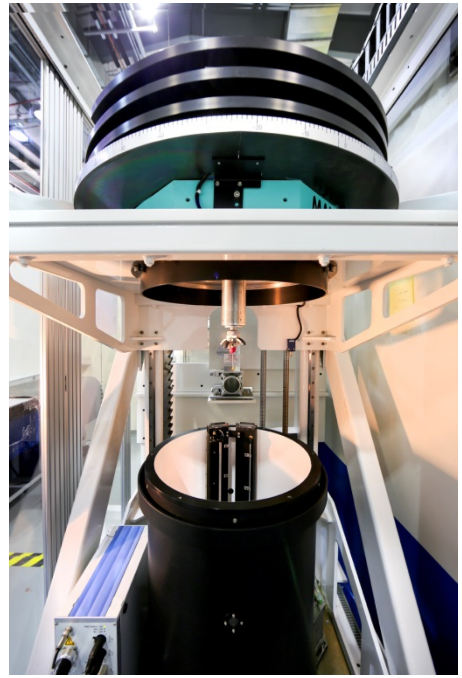
IMAGINE – Beam line overview

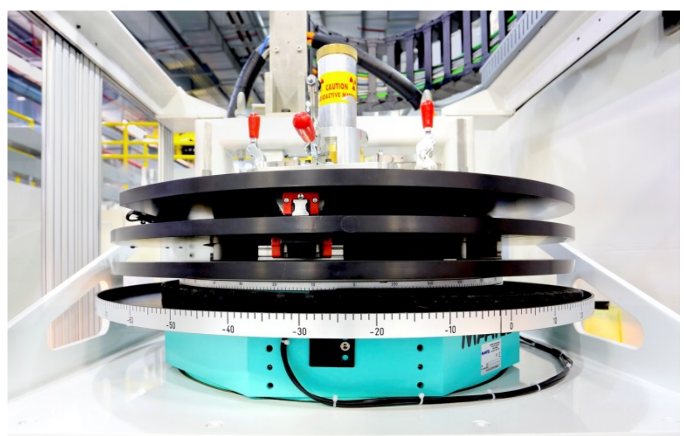
At the sample position:
Flux: ~3 x 10^7 n s^{-1} cm^{-2} (2.8 Å – 4.0 Å)
Beam size: 2 x 3.2 mm
Divergence: ~0.6º





IMAGINE – Image Plate Diffractometer



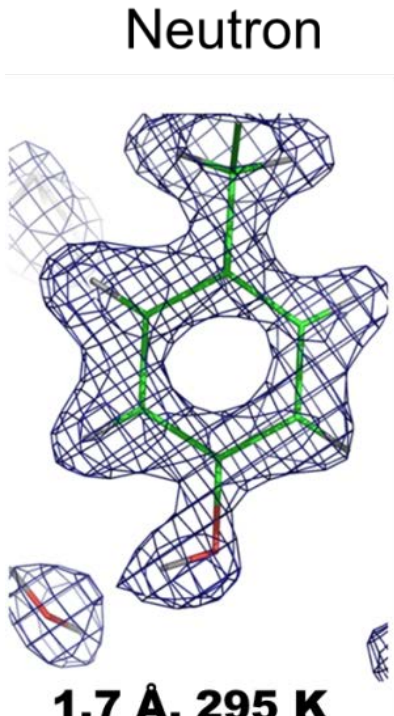


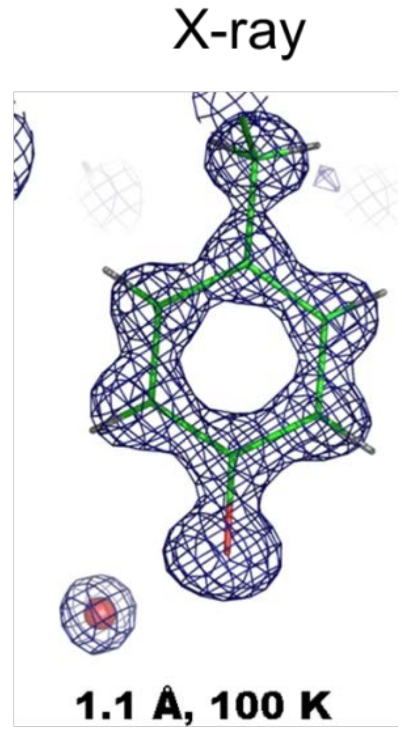
Detector	Neutron image plate
Detector size	1200 x 450 mm
Pixel size	125, 250, 500 μm
Sample-to-detector distance	200 mm
Goniometer	Single Phi rotation axis

http://neutrons.ornl.gov/imagine/

Neutron protein crystallography

- Visibility of hydrogen/deuterium atoms
- No radiation damage





Gardberg *et al.* (2010) *Acta Cryst.* D**66**:558-567

NPX is a unique experimental tool for the experimental location of key hydrogen atoms and water molecules in biological macromolecules.

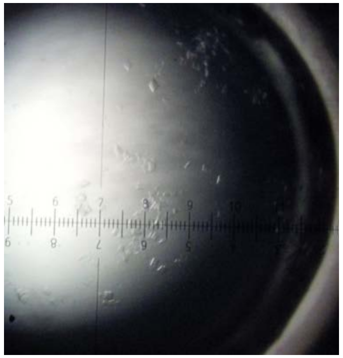
Sample preparation: Biodeuteration Laboratory

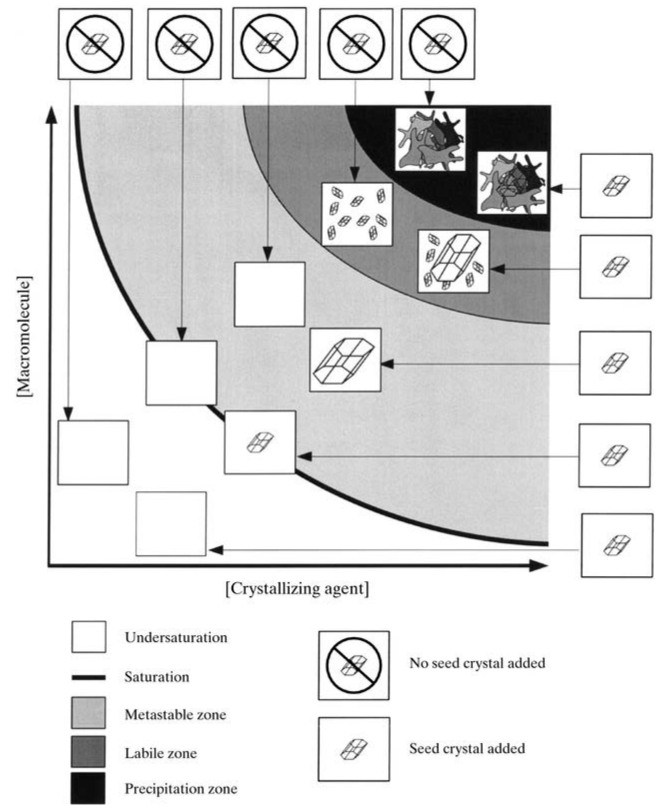
- Perdeuteration:
  - Increases signal / noise ratio
    - Incoherent scattering from D is 40 times lower than incoherent scattering from H
    - Background of quasi-Laue experiments is “high”

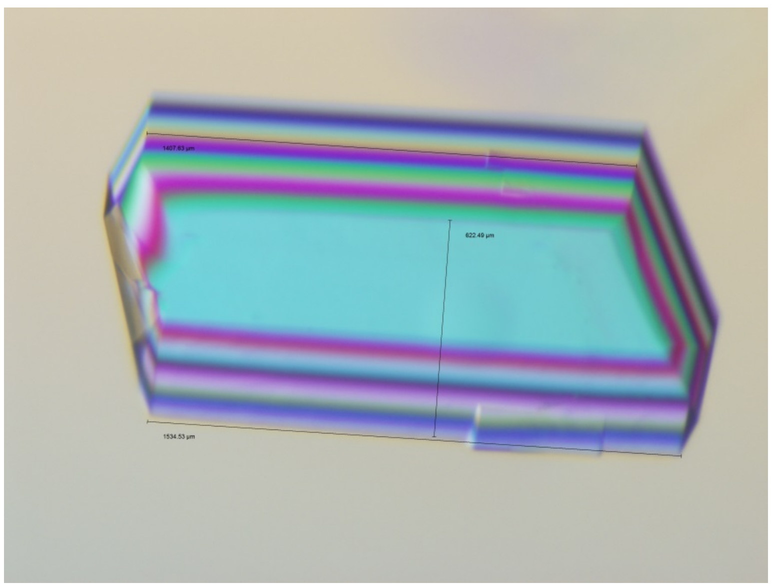
    > Smaller crystals can be used

    and/or data collection time can be reduced
  - Enhances the visibility of H position in the neutron maps (no cancellation)

Sample preparation: Crystallization Laboratory

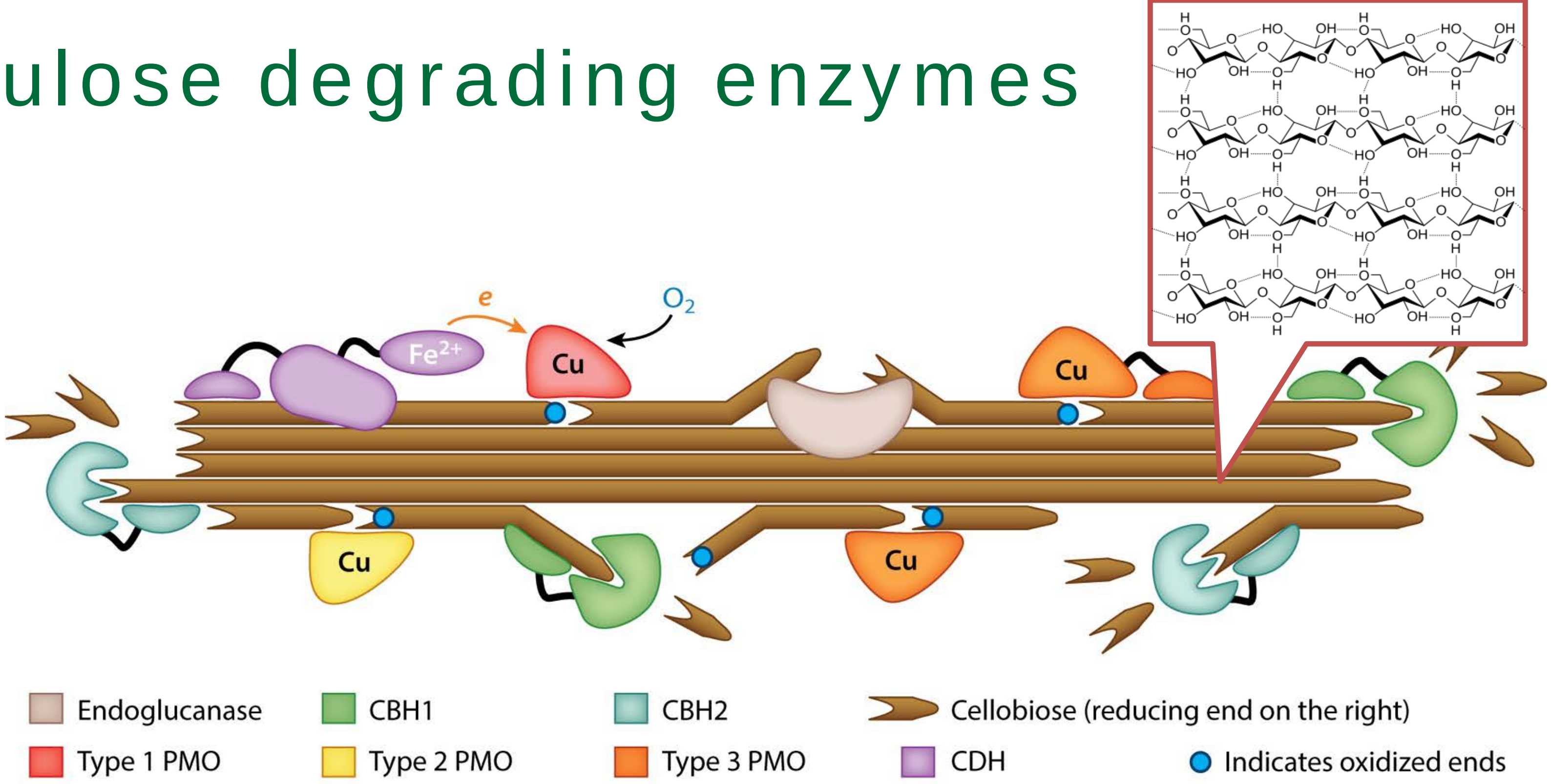






Luft & DeTitta (1999) *Acta Cryst* D**55:**988-993

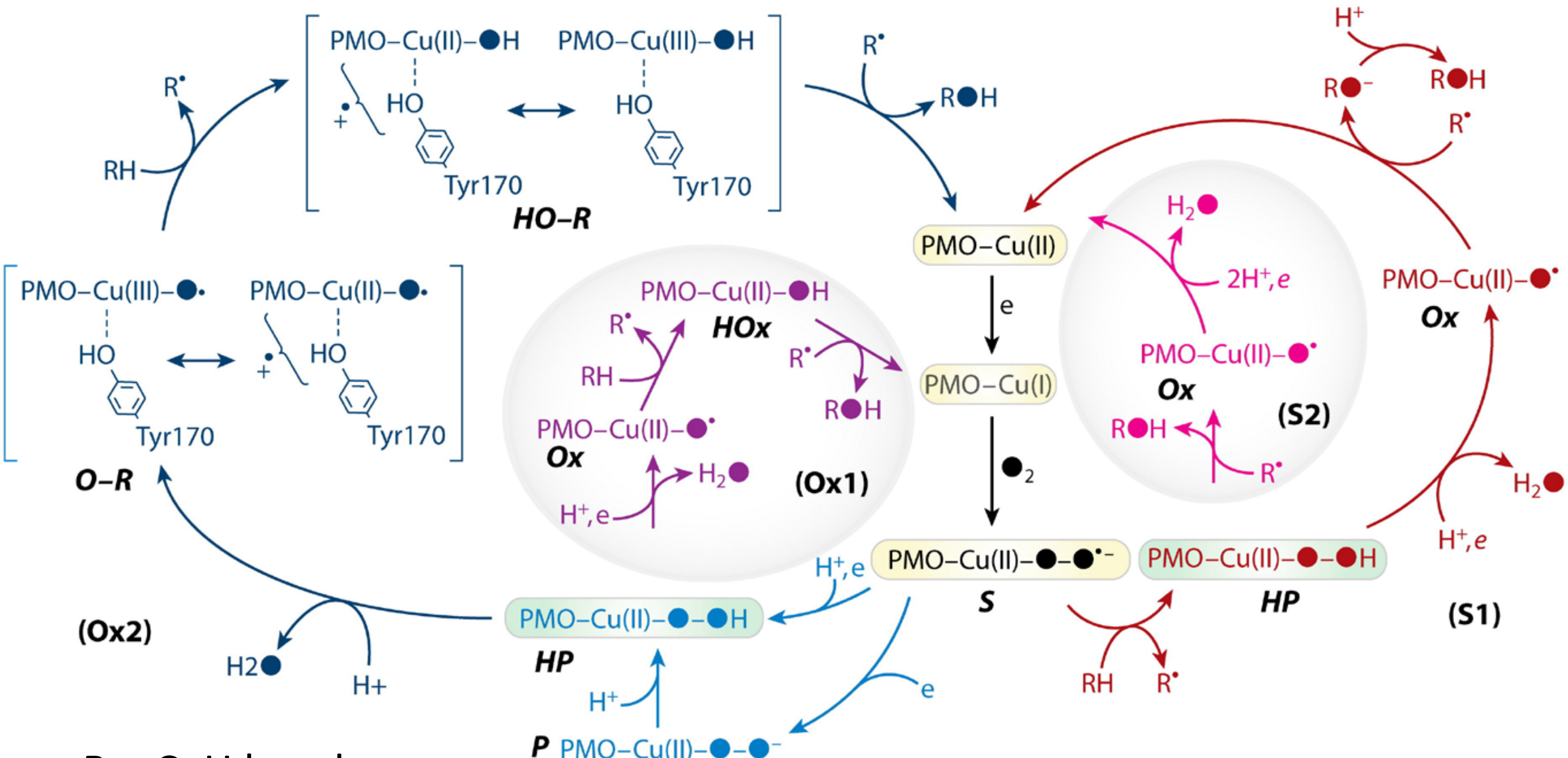
Cellulose degrading enzymes



Beeson *et al.* (2015) *Annu. Rev. Biochem.* **84**:923-946

- A variety of enzymes function together to break down cellulose to soluble sugars.
- Two important enzymatic activities
  - Hydrolytic (Endoglucanases, CBHs)
  - Oxidative (LPMOs, CDHs)

Possible LPMO reaction mechanisms

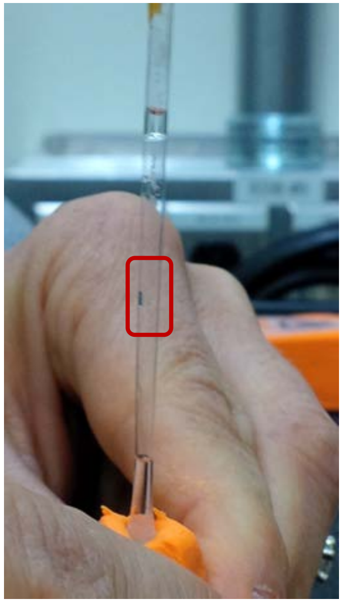


R = C–H bond

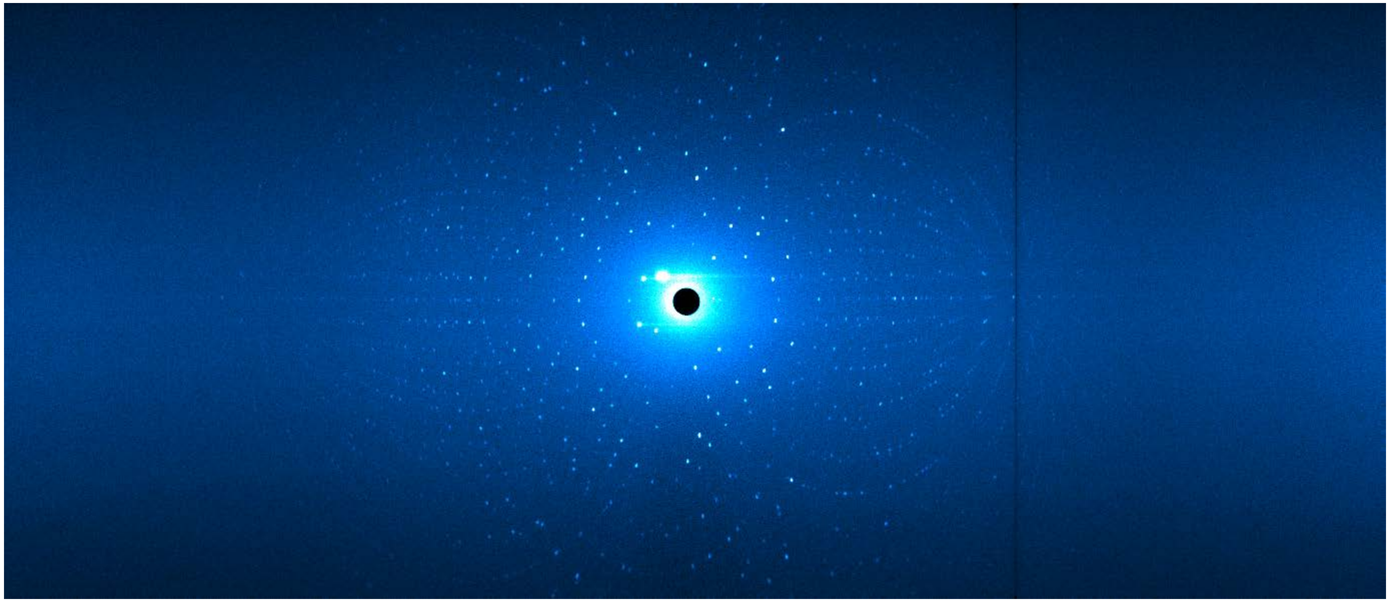
Beeson *et al.* (2015) *Annu. Rev. Biochem.* **84**

*Nc*LPMO9D neutron diffraction

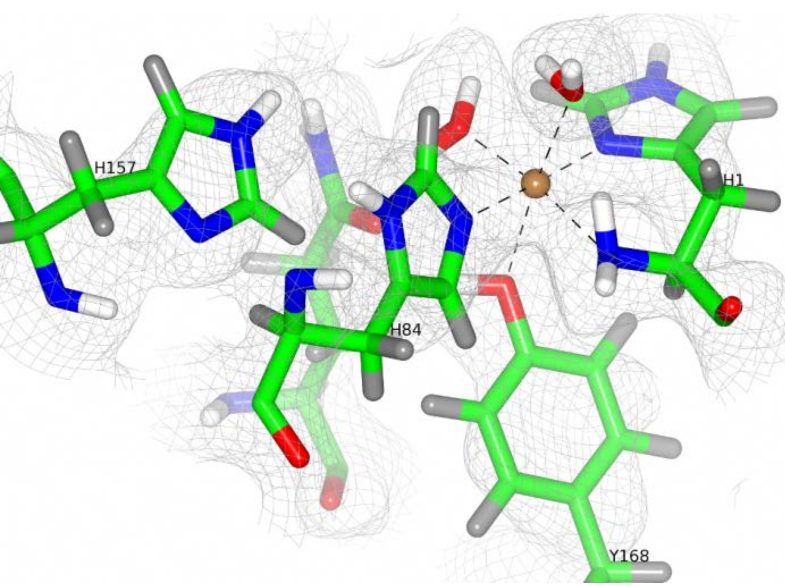
- ~0.33 mm^3 crystal
  - Natural isotopic abundance
  - Vapor exchange against ^2H_2O artificial mother liquor



- Quasi-Laue diffraction data
  - λ = 2.8 – 4.5 Å; Exposure: 24 hr / frame; # of frames: 21
  - d_{min} = 2.1 Å

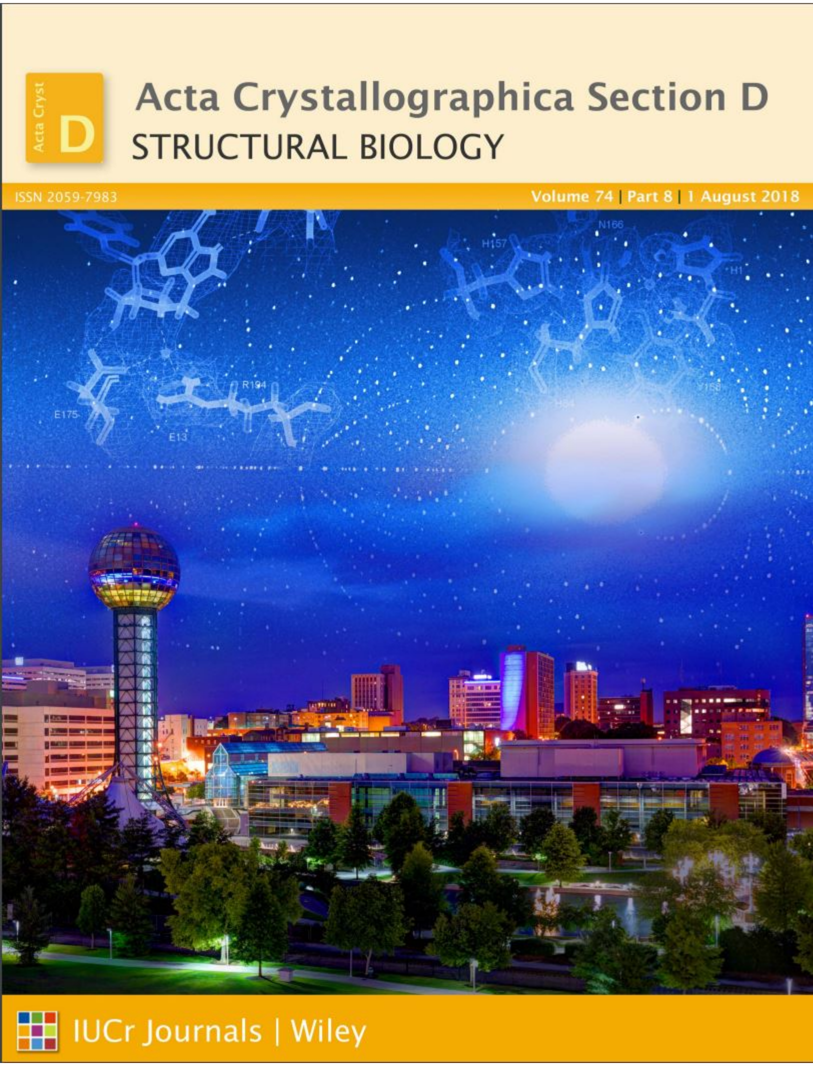


cture, resting state

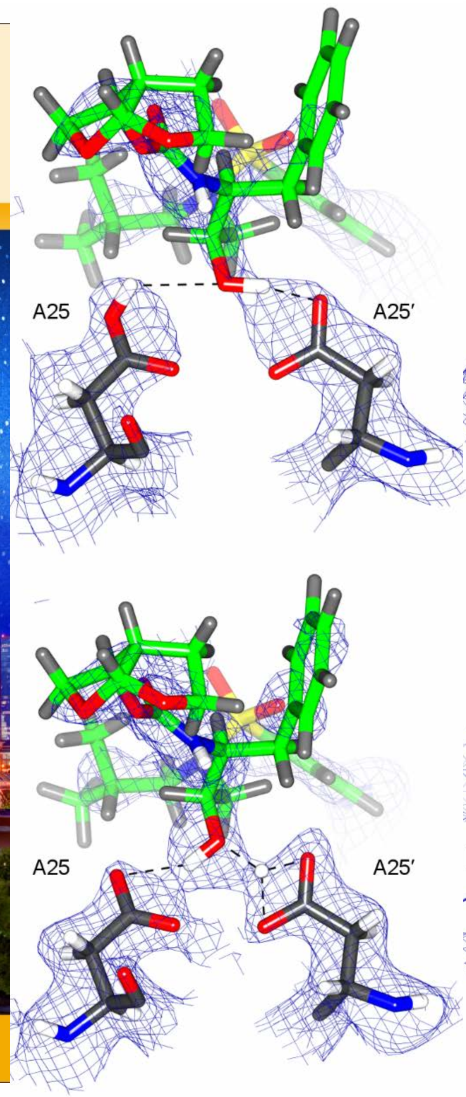


O'Dell, Agarwal, Meilleur (2017) *Angew. Chem. Int. Ed.***56***:767*

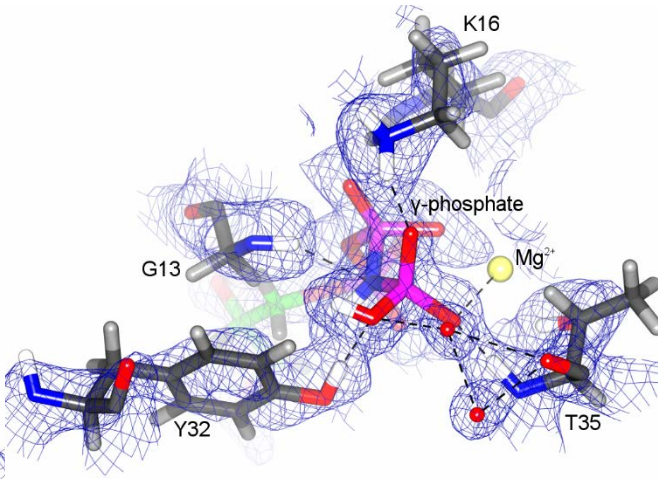
Celebrating 5 years in the User Program



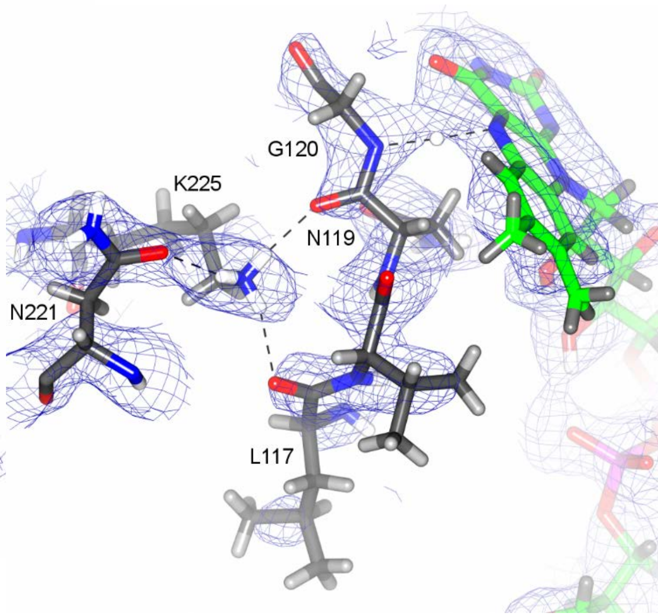
Schroder *et al.* (2018) *Acta Cryst.* D**74**, 778-786



HIV protease



RAS



Cholesterol Oxidase

Magnetic studies on IMAGINE

Magnetic system studies at low-temperatures ($TbNi_2Ge_2$)

Team: Rob McQueeney, Karunakar Kothapalli, Scott Saunders (Ames), Bryan Chakoumakos, Lakeisha Walker, Flora Meilleur

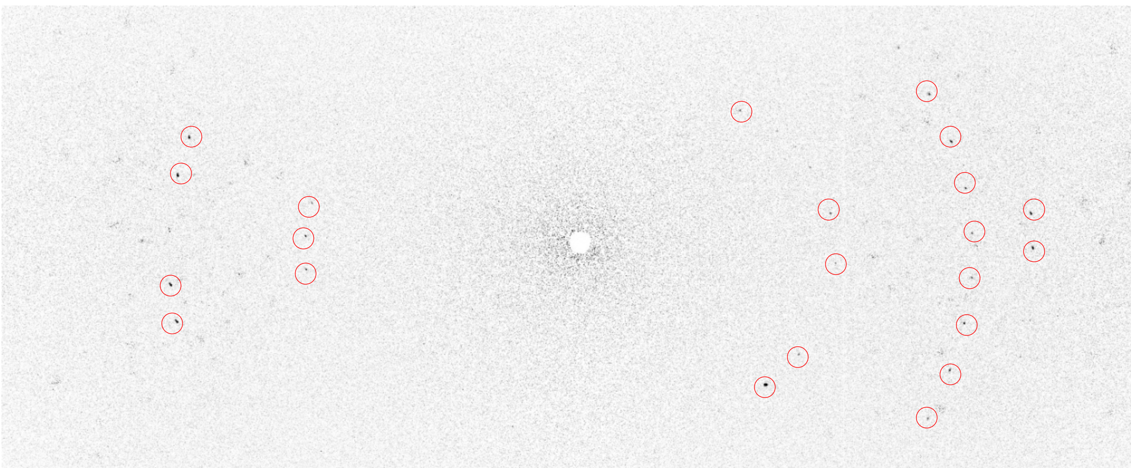


Figure (Top): Subtraction of a diffraction pattern collected at 20K from a diffraction pattern collected at 4K. Red circles highlight the magnetic super lattice peaks.

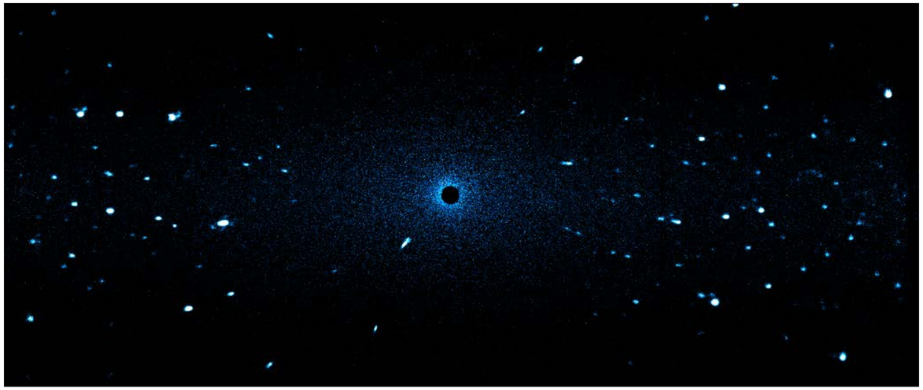
Figure (Left): Graduate student Scott Saunders mounts a sample with IMAGINE SA Lakeisha Walker.

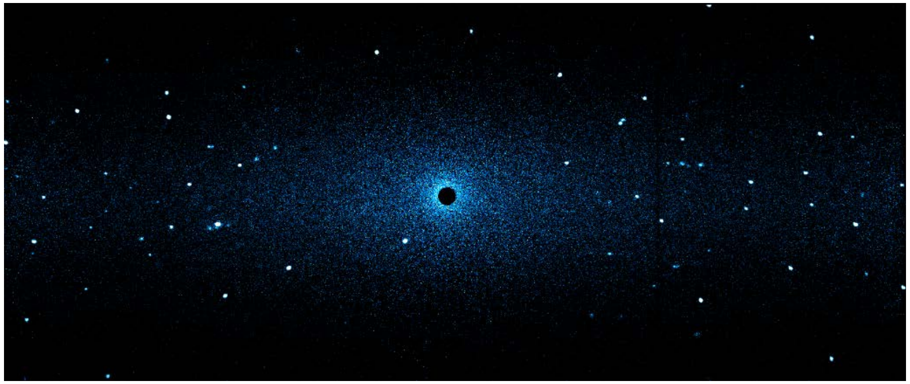
Structural transition on IMAGINE

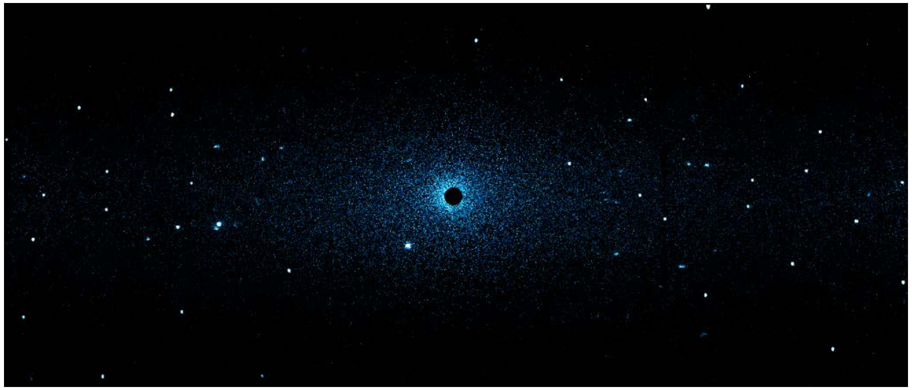
Structures of organic-inorganic “hybrid” perovskites

Data collection at 10K (top), 270K (middle) and 400K (bottom) to track structural transition;
12 hours per complete data set

Team: Huibo Cao, Bryan Chakoumakos, Lakeisha Walker, Flora Meilleur







IMAGINE

- Rapidly tunable
- High intensity
- Versatile
- Sub-millimeter crystals (still very large for bio. applications $\rightarrow$ DNP)
- Large unit cells
- Extreme environments (temperature, pressure)

Acknowledgement

IMAGINE team

Bianca Haberl