Structure and Dynamics of Biological Systems

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Pacific Northwest NATIONAL LABORATORY
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Project 1.8; October 2012 – September 2015

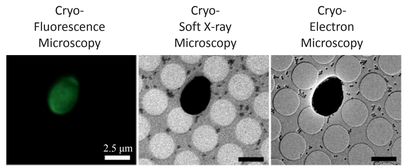
Purpose

- Overcome obstacles to linking biological systems' structure to their physiological function caused by system complexity and the wide range of spatial and temporal scales (femtoseconds to hours and angstroms to meters) over which their dynamic processes occur
- Generate a multimodal, multi-scale imaging suite integrating atom-probe tomography (APT), in situ time-of-flight and nano- secondary ion mass spectrometry (SIMS), nano- to microsecond dynamic transmission electron microscopy (DTEM), and ultrafast X-ray diffraction at the Linac Coherent Light Source (LCLS)

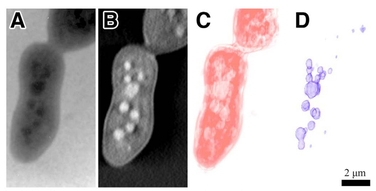
Key Successes

- 6 published or in press papers
- 2 submitted manuscripts
- 8 invited presentations
- 6 contributed talks/posters
- EMSL user proposal awarded
- 3 LCLS user proposals submitted
  - 2 awarded, 1 in review
  - 108 hours awarded in FY13

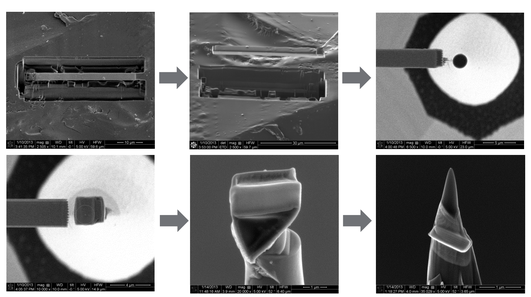
Research Accomplishments



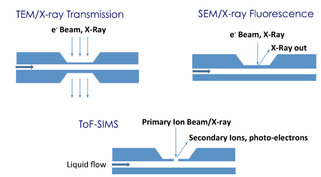
Multimodal correlative 2D imaging of the exact same cell.
Carlson DB et al. 2013. *Microscopy & Microanalysis* 19(1):22-29



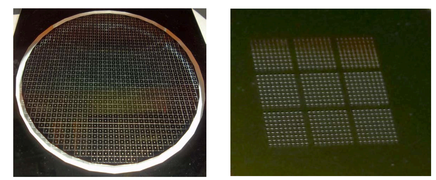
Whole-cell 3D X-ray nanotomography without staining or sectioning.
Evans JE et al. 2013. *BioOptics World* 6(2):28-39.



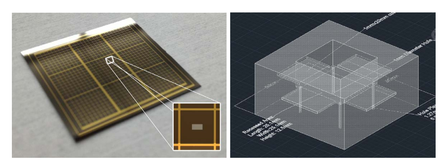
FIB preparation of Lowicryl resin with embedded sample. Lowicryl resin lacks N and P allowing unambiguous identification of the embedded protein.



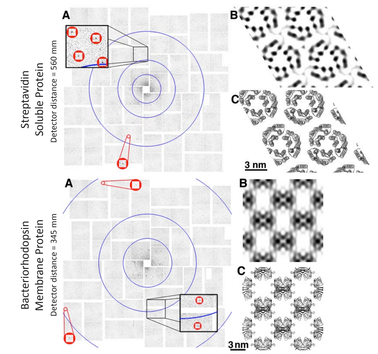
Geometry of in situ liquid environmental cells by imaging technique.
Yu XY et al. 2013. *Microfluidics and Nanofluidics*, In press.



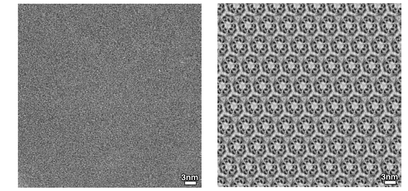
Onsite fabrication of in situ liquid cells for TEM, X-ray, and SIMS.



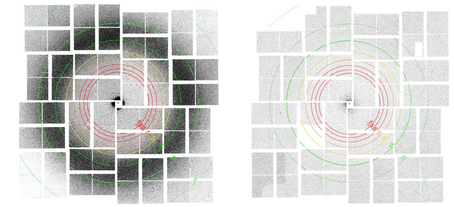
In situ liquid cell with 729 isolated windows for pump-probe and hydrated ultrafast X-ray diffraction and schematic of sealing jig.



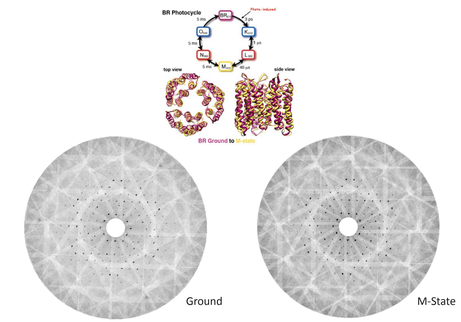
First transmitted 50-fs X-ray diffraction from 2D protein crystals. Blue = Resolution rings at 30, 15, and 7 Å (inner to outer).
Frank M et al. 2013. *Nature Communications*, submitted.



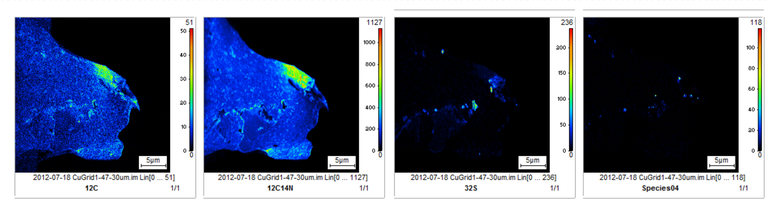
Cryo-EM (left) of bacteriorhodopsin 2D crystal and resulting density map (right) correlating with above X-ray diffraction data.



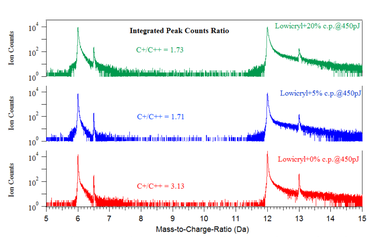
X-ray diffraction from 2D protein crystals sugar embedded and in vacuum (left) versus fully hydrated within in situ cell (right).



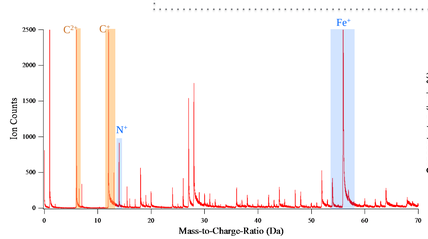
Bacteriorhodopsin's photocycle (top) is ideal for initial pump-probe dynamic studies at LCLS (left and right) and with Dynamic TEM (FY14).
Evans JE and ND Browning. 2013. *Microscopy* 62(1):147-156.

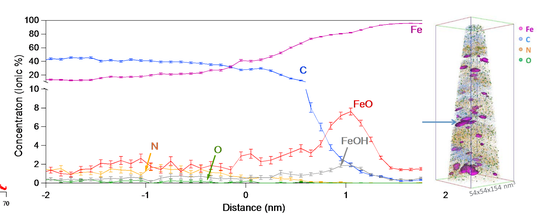


Nano-SIMS generates elemental maps of microbial biofilms on TEM support grids for correlative microscopy.



0-20% conductive polymer doping of Lowicryl resin (insulator) enhances spectra by improving ratio of detected C^+/C^{2+}.





APT of Lowicryl resin with embedded ferritin (macromolecule containing a protein shell and iron oxide core). Use of conductive polymer doped Lowicryl has permitted first ever APT mapping of protein composition relative to the resin background.

Next Steps

Pursue external funding opportunities:

- DOE-BES Early Career Award
- NIH RO1 grant

FY14 New Research Areas:

- Assess DTEM's diffract-and-destroy compatibility for cryo-EM using µs-ns single-shot electron pulses
- Acquire movies of biological processes in real-time including conformational changes upon ligand binding and assembly mechanisms
- Adapt nano-SIMS for robust serial section analysis of biological systems and correlative microscopy
- Design and fabricate new in situ liquid cells for X-ray, electron, ion, and optical imaging
- Implement cryo-transfer system for APT

PNNL-SA-96602