Argonne NATIONAL LABORATORY

LIGHT-SOURCE-BASED X-RAY IMAGING FOR BIOLOGY: FROM TRACE ELEMENTS TO CELLULAR STRUCTURE

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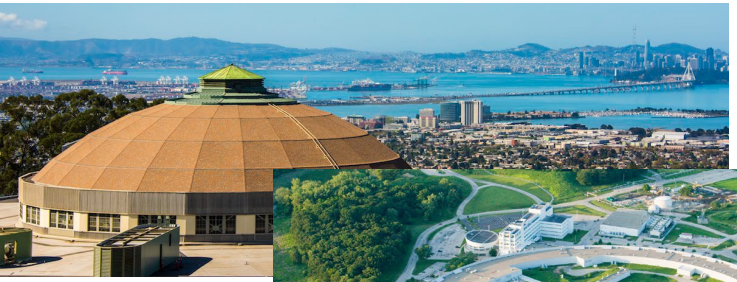
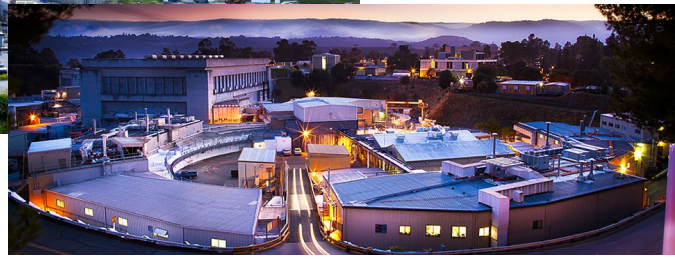
ACKNOWLEDGEMENTS





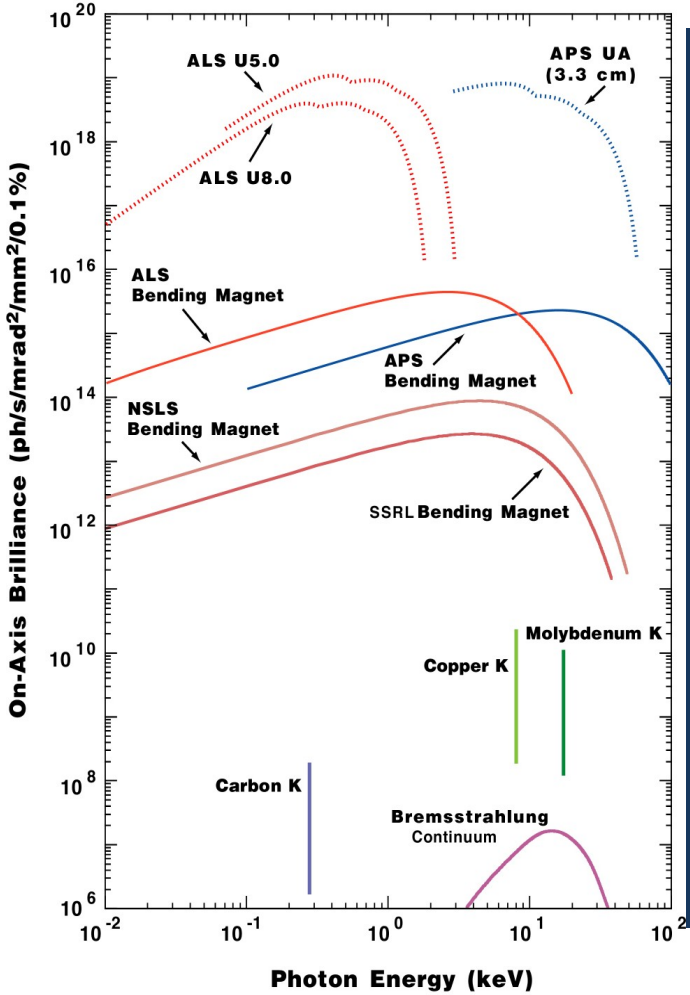
Teams from the 5 DOE light sources
Numerous users
Sponsors: DOE BES & BER, NIH institutes, …

STORAGE RING AND FELS

Storage Rings

Near continuous sources with high average brightness, wide tunable energy range and high stability enable:

- **Imaging** processes on the 100ps – days timescale
- Balanced flux on sample to follow processes (interact but don't destroy)
- Diverse, highly optimized, multiplexed endstations for a wide range of scientific communities and numerous user groups



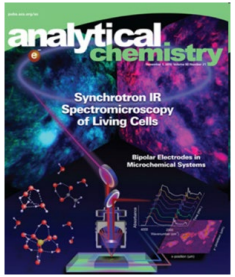
Free Electron Lasers

Pulsed sources with ultra-high peak and average brightness with full spatial coherence enable:

- Pump probe: Resolving ultrafast processes (<=ns)
- Near-instantaneous snapshots of processes in isolated areas ('diffract before destroy')
- A small number of endstations addressing carefully selected, high profile problems

INFRARED SPECTROMICROSCOPY

INFRARED SPECTROMICROSCOPY



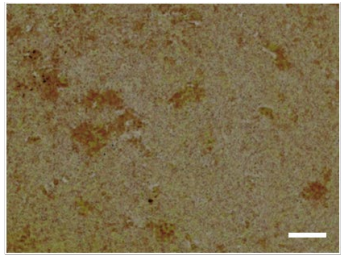
Anal. Chem, 82, 8757 (2010)

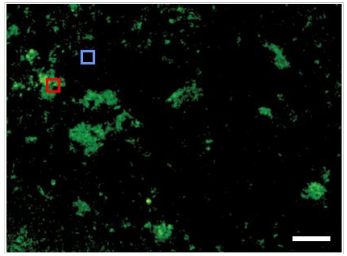
Chemical Imaging of Biological Systems

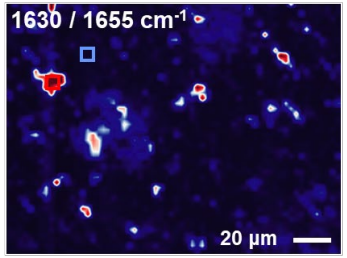
- Biogeochemistry and Environment
- Health and Medicine
- Bioenergy
- Interfacial Phenomena

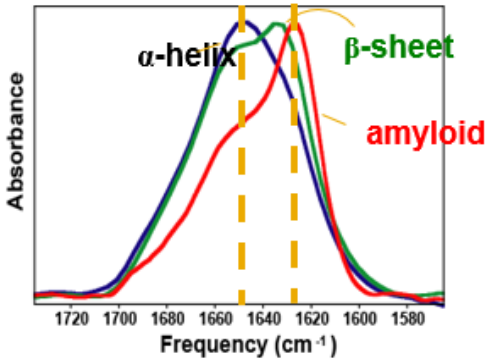
Micro-spectroscopy

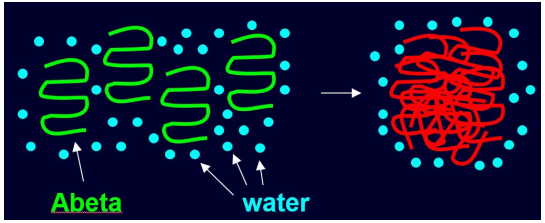
- 2-10 um spatial resolution, diffraction limited











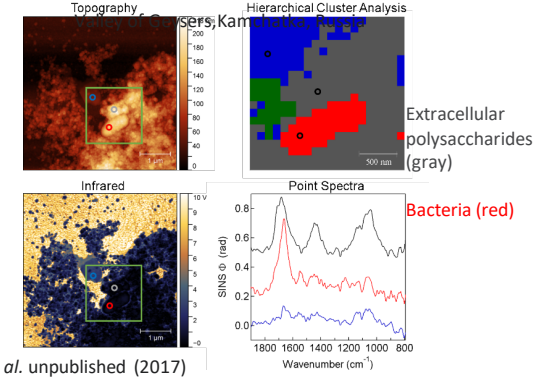
- Alzheimer's disease is characterized by the accumulation of plaques in the brain.
- Plaques are misfolded Abeta protein.
- IR microspectroscopy can image misfolded proteins within tissue based on increased amyloid (β-sheet) structure.

L.M. Miller, et al., *J. Struct. Biol.*., 155 (1): 30-7 (2006).

Nano-spectroscopy

- < 25 nm spatial resolution, wavelength independent

Microbial Biofilms in Boiling Hot Springs



Holman *et al.* unpublished (2017)

work done at ALS

Hoi-Ying Holman
hyholman@lbl.gov

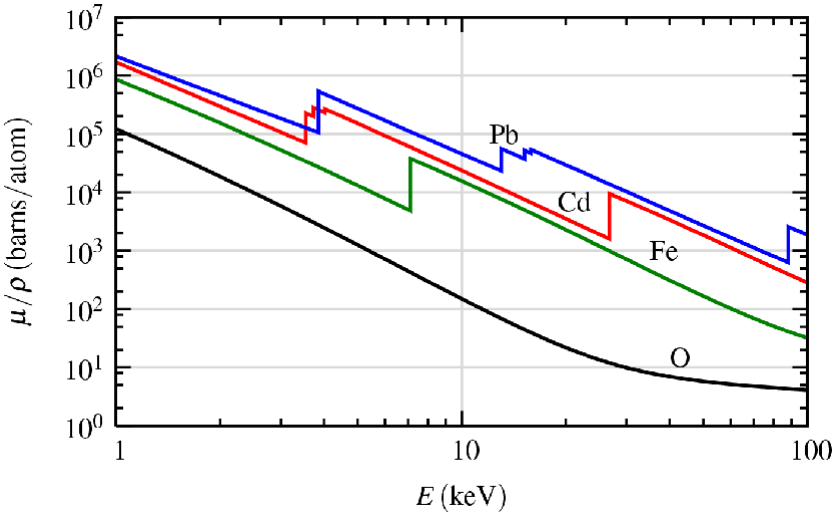
work done at NSLS

X-RAYS

X-RAY ABSORPTION EDGES

X.rays have energies comparable to *binding energies* of electrons in atoms

As you cross an absoprtion edge, x-rays have enough energy to kick out another bound electron, and absorption increases significantly



Some Binding Energies (eV)

H 1s	13.6
O 1s	545
Fe 1s	7112
Pb 1s	88005
Pb 2p3/2	13043

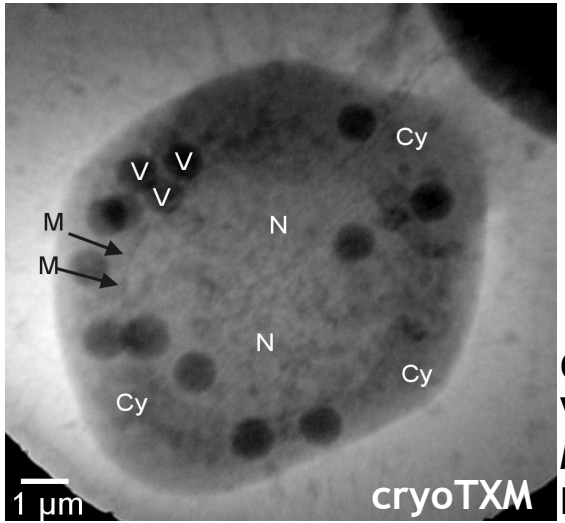
Enables:

- **Tuning the contrast for detecting a particular element.**
- **Spectroscopy to detect chemical state**

Courtesy M. Newville

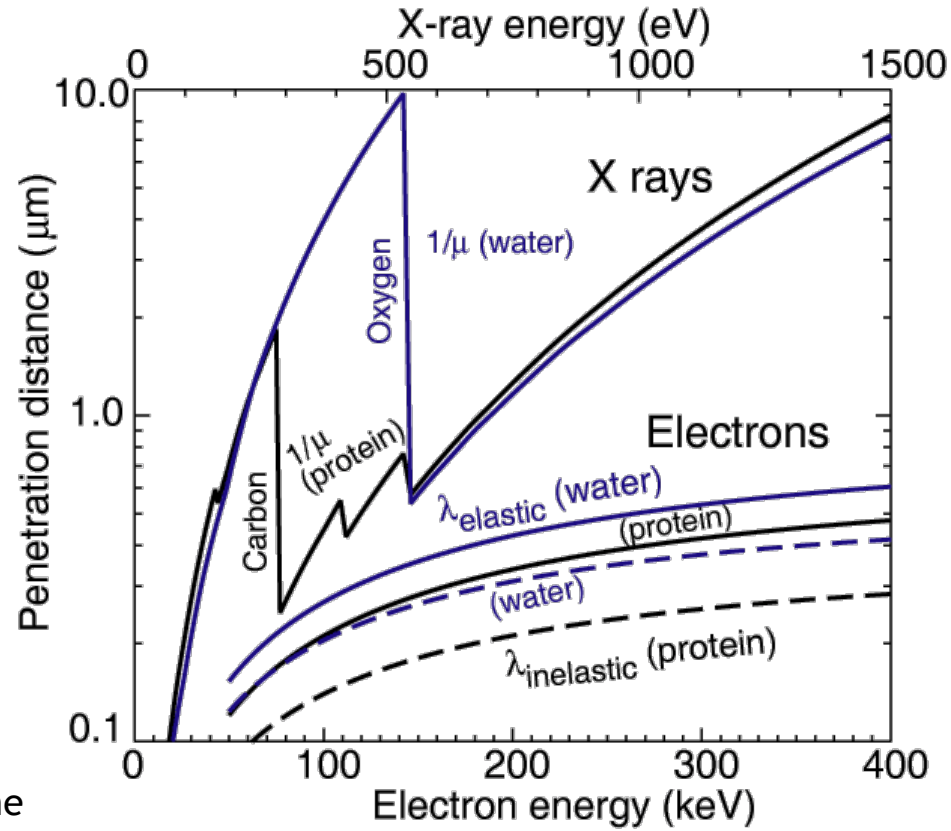
TRANSMISSION X-RAY MICROSCOPY (SOFT X-RAYS)

- Natural contrast between protein and water in the so called water window
- Spatial resolution not limited by wavelength



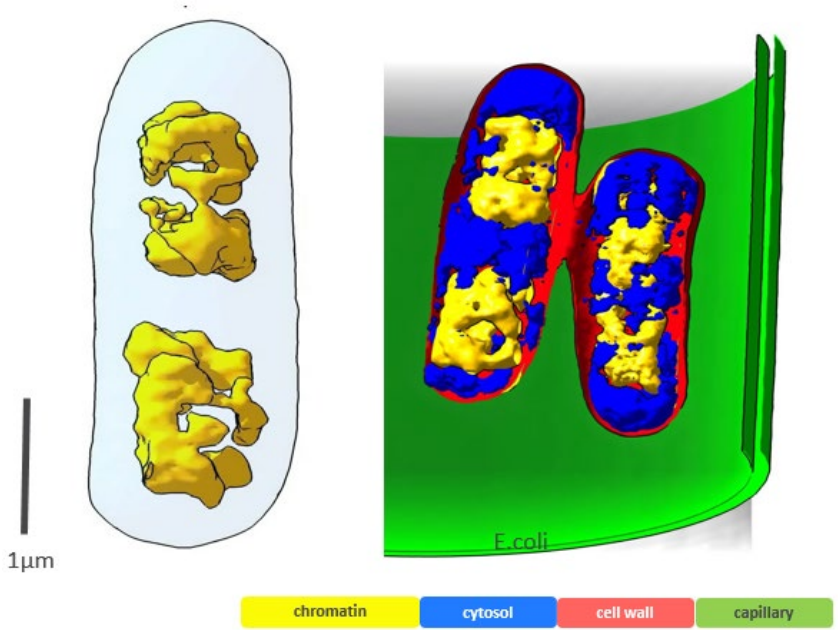
- *Drosophila melanogaster* cell, in vitrified ice, imaged @ 0.5 keV @ BESSY

Cy: cytoplasm
V: vesicle
M: nuclear membrane
N: nucleus

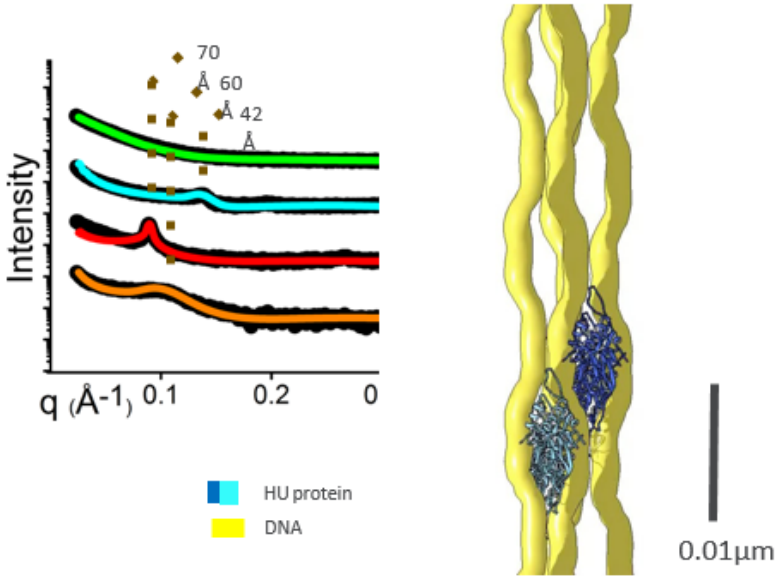


Solution X-Ray Scattering and X-Ray Tomography Revealed Bacterial-Chromatin Packing Across the Nanoscale and Mesoscale

Soft X-ray Tomography (SXT)



Small Angle X-ray Scattering (SAXS)



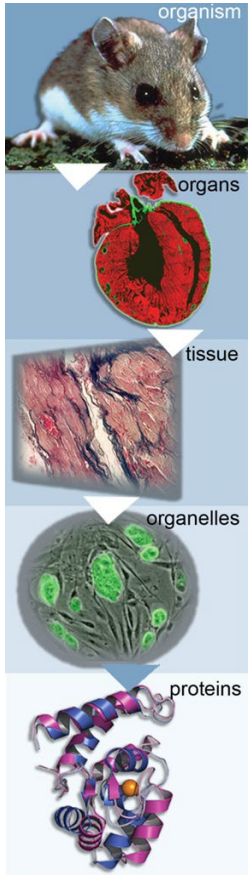
1.HU multimerization shift controls nucleoid compaction.
Hammel M et al. Science Adv. 2016;2(7):e1600650
2.Nuceloid remodeling during environmental adaptation is regulated by HU dependent DNA bundling
Remesh SG et al. 2020 Nature Communications (in revision)

National Center for X-ray Tomography
Supported by NIH-NIGMS and DOE-BER

The SIBYLS Beamline
brought to you by IDAT, SBDR, and the DOE

work performed at ALS

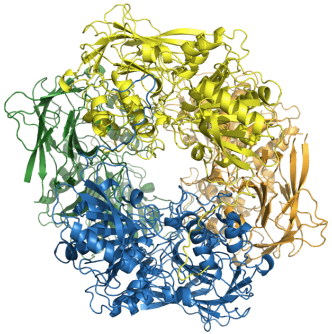
HARD X-RAYS

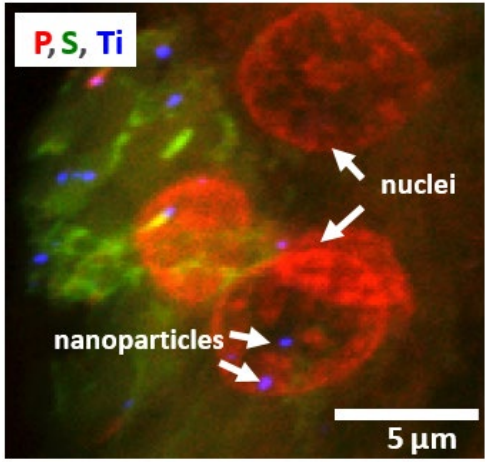


BIO IMAGING WITH HARD X-RAYS

- **Diffract and scatter**
  - Structure determination
- **Imaging and Tomography**
  - Hard x-rays penetrate matter deeply
  - Visualization of structure in ‘thick’ samples (>1um), over large field of view
  - Live imaging possible at reduced resolution
  - Nanotomography down to 20 nm 3D resolution, lensless imaging down to 10 nm resolution
- **(Trace) Element Imaging via X-ray Fluorescence**
  - Quantitative ion distributions at physiological concentrations, metal homoestasis, metal-linked diseases …
  - Therapeutic (metal-based) drugs and diagnostic agents, theranostics, …
  - Low background, no labeling required
  - Visualization of chemical state
- **Radiation Damage Mitigation:**
  - Cryogenic temperatures
  - Reduction of X-ray dose

(K. Michalska et al. 2015)

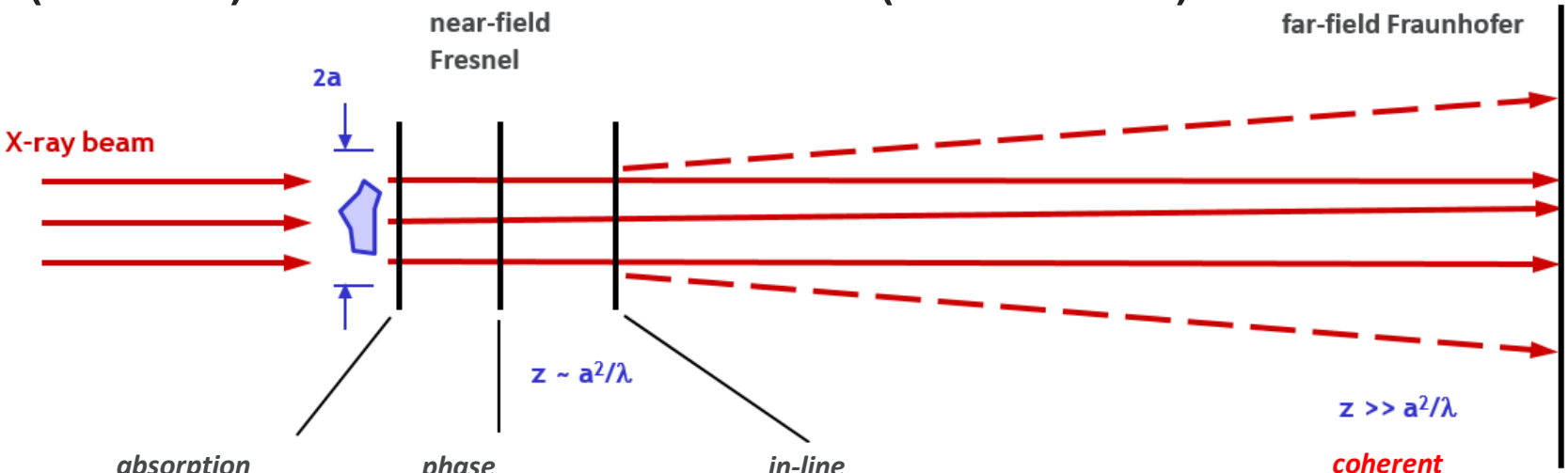


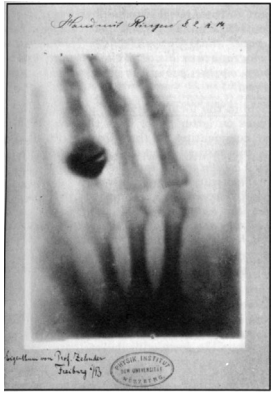


Uptake of TiO_2 nanoparticles (blue) into liver tumor cells one hour after injection. Sulfur (green) indicates cytoplasma, phosphorous (red) DNA.
(Part of NCI Cancer Close Up 2017 https://visualsonline.cancer.gov/)

(DIRECT) IMAGING REGIMES WITH (COHERENT) X-RAYS

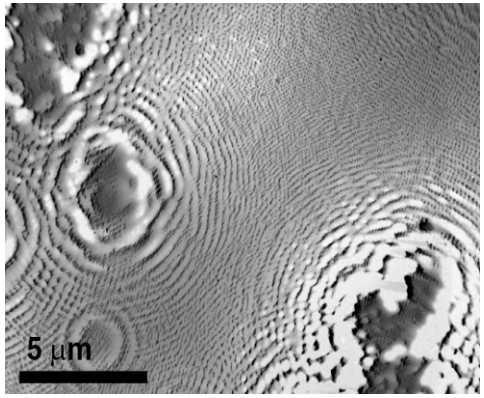
Adapted from Prof. Oleg Shpyrko (UCSD)



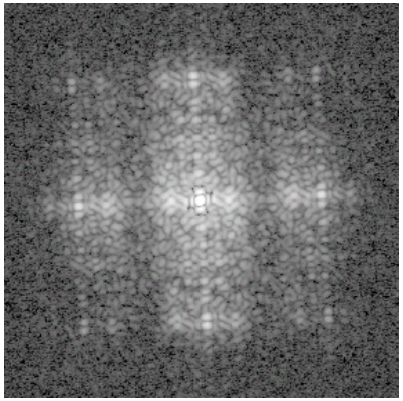


Roentgen (1895)

Kagoshima (1999)



Jacobsen (1990)



Miao (1999)

X-ray *in vivo* microtomography of embryonic evolution

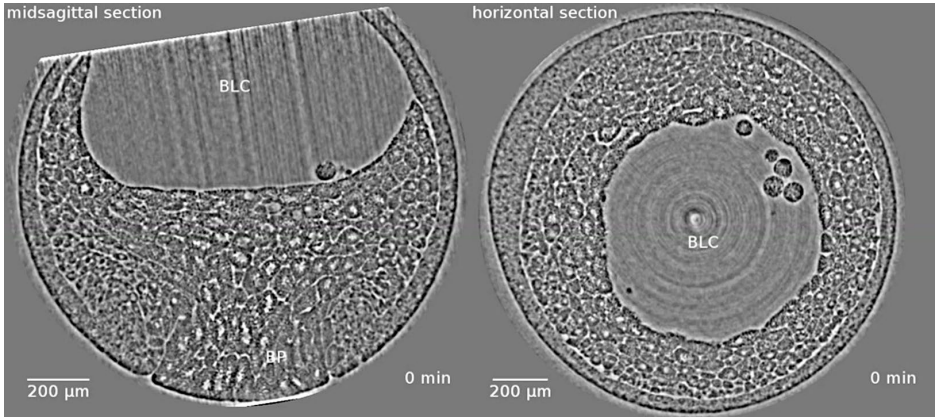
During gastrulation: series of dramatic, coordinated cell movements drive reorganization of a simple ball or sheet of cells into a complex multi-layered organism.

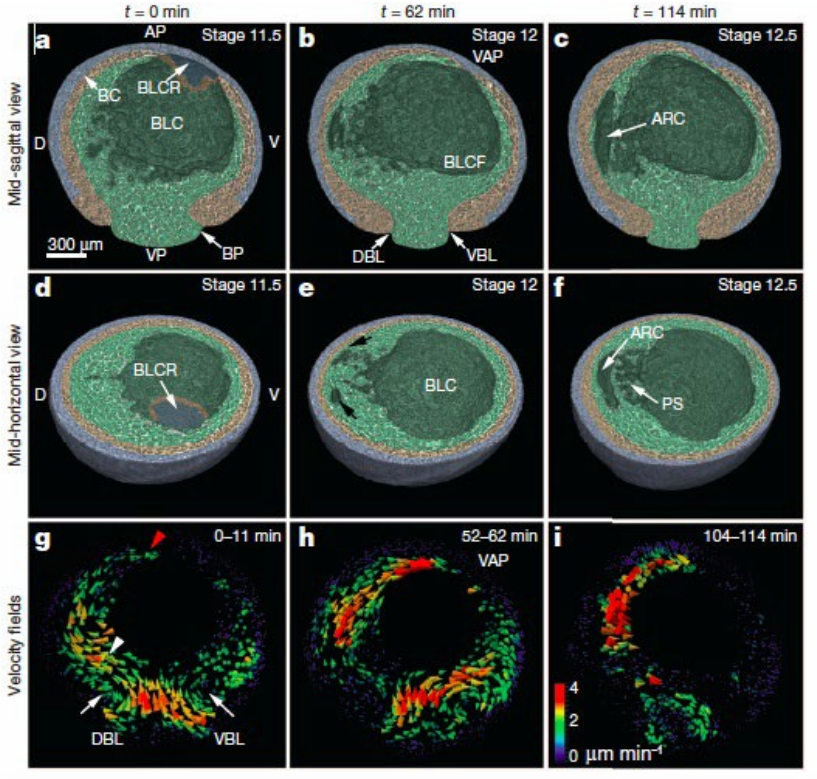
Scientific Goal:

Understand the behavior of cells during development by imaging—in vivo and with subcellular resolution.

Method:

Phase contrast image before damage, Series of tomograms every 10 min, Tomogram = 1200 projections in 18 s



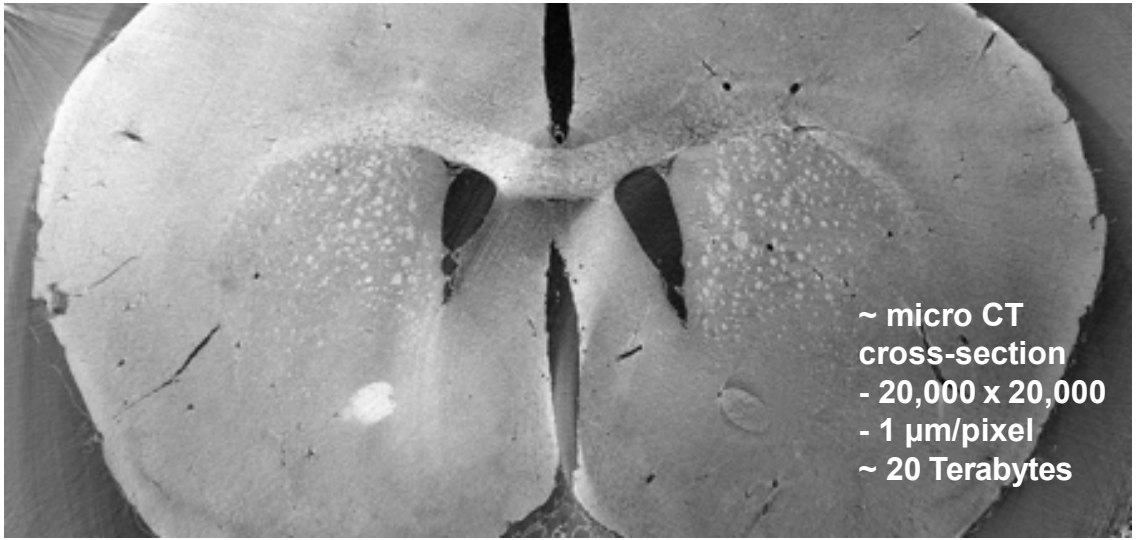


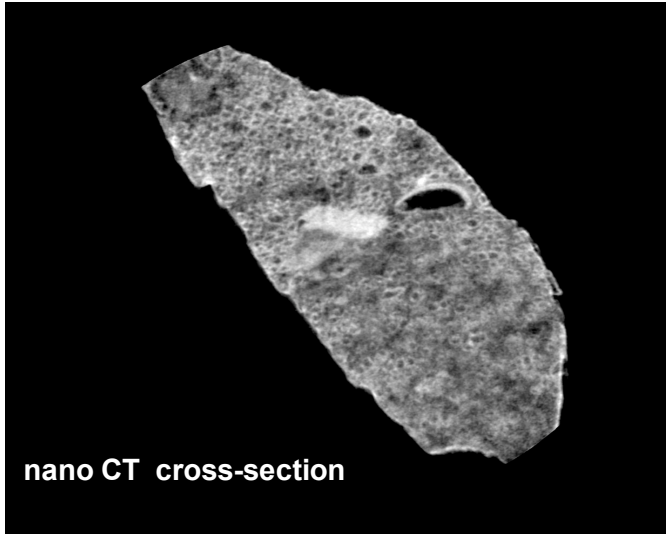
3D time-lapse series of X. *laevis* embryo during mid-gastrulation

J. Moosmann et al., Nature 497, 394 (2013)

work done at APS

Brain Imaging





Brain mapping currently occurs at orders of magnitude disparate resolutions and volumes from nanometer reconstructions of small volumes of brains with electron microscopy to mm^3 voxel resolution maps of whole brains with MRI. Large gap remains in our understanding of brain anatomy at the mesoscale – detailing the cellular compositions of entire brains along with the trajectories of the vasculature and the long distance projections of neurons between and within brain regions. X-rays today can bridge the gap between those lengthscales.

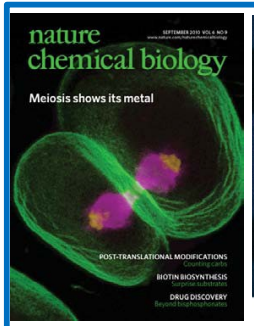
- tomographic cross-section of a full mouse brain at 1 μm resolution (left: one extracted cross section of a full 3D dataset)
- nano-tomographic datasets down to ~30 nm resolution (right) and 10s of um field of view

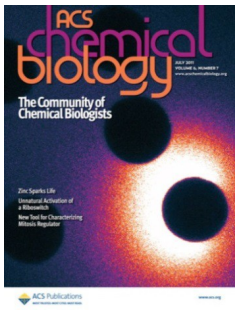
Planned upgrades and developments are expected to extend technique to image at ~10 nm 3D resolution across mm sized volumes.

work done at APS

IMAGING FUNCTION: ELEMENTAL CONTRAST - TRACE METALS IN THE LIFE SCIENCES

- Trace elements (metals) are fundamental, intrinsic components of biological Systems. estimated: 1/3 of all known proteins contain metalcofactors as integral, catalytic components, often with regulatory functions, e.g.,
  - Zn in Zinc finger proteins: transcription factors
  - Fe in Haemoglobin; and necessary in Chlorophyll synthesis
- Metals are linked to diseases
  - Endogenous dysregulation, e.g., Alzheimer's, ALS, Wilson disease (Cu accumulation)
  - Exogenous uptake, e.g., Pb, As, Hg (or lack thereof: e.g., Se deficiency)
  - Bio-remediation
- Metals in therapeutic drugs and diagnostic agents
  - Cis-platin in chemotherapy
  - Gd in Magnetic resonance imaging (MRI)
  - Novel bio-inorganic nanoparticles, in particular Nanomedicine: multifunctional nanovectors ideally combining targetting, therapy (e.g., Pt, TiO2) and diagnosis (e.g., Gd)



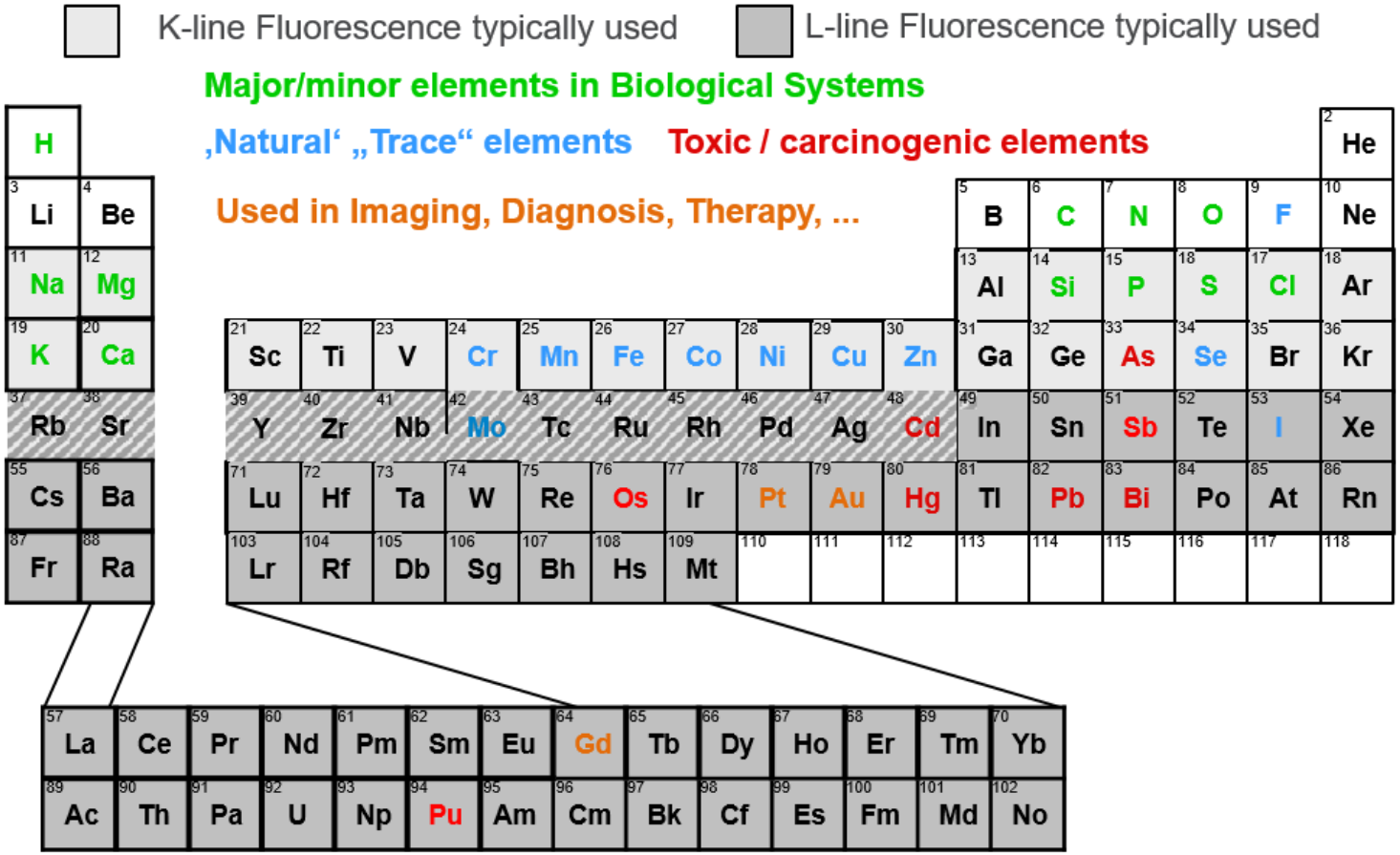


Zinc plays an unexpected role in oocyte maturation: Kim *et al*. Nat Chem Biol. 2010 **6**(9):674-81; Kim et al, ACS Chem Biol, 2011. **6**(7): p. 716-23.

Reviews of **XFM applications**:
Imaging: T. Paunesku *et al*., J Cell Biochem **99**(6), 2006
Spectroscopy: C. Fahrni, Curr Opin Chem Biol **11**(2), 2007

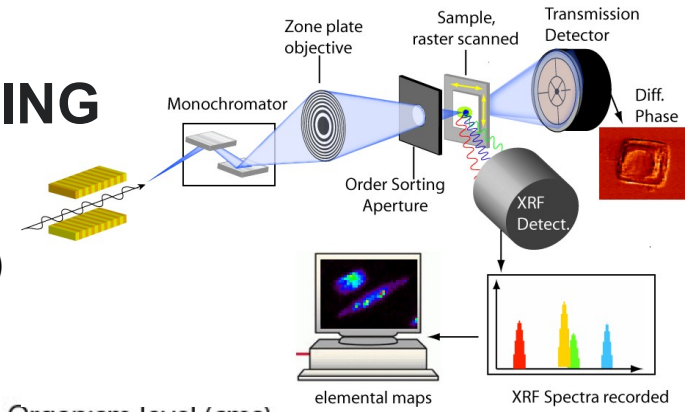
Review of **XFM tomography**:
M. de Jonge & S. Vogt, Curr Opin Struct Biol **20**(5), 2010

Periodic table highlighting X-ray fluorescence

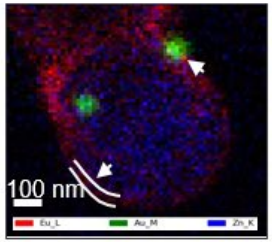


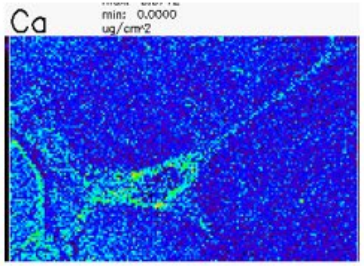
SCANNING PROBE IMAGING: X-RAY ABSORPTION SPECTROSCOPY IMAGING

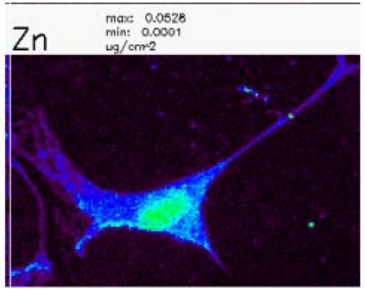
- *Provide world-class micro-to-macro XAS/EXAFS spectroscopy capabilities with elemental mapping and imaging – enables speciation information*
- Facilities cover a wide range of energies (2-25 keV) and spot sizes (2-500 µm) to meet requirements of a wide variety of science

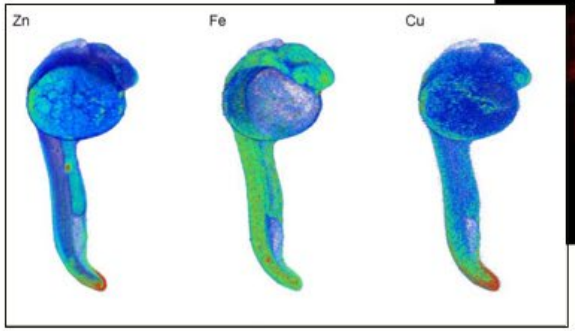


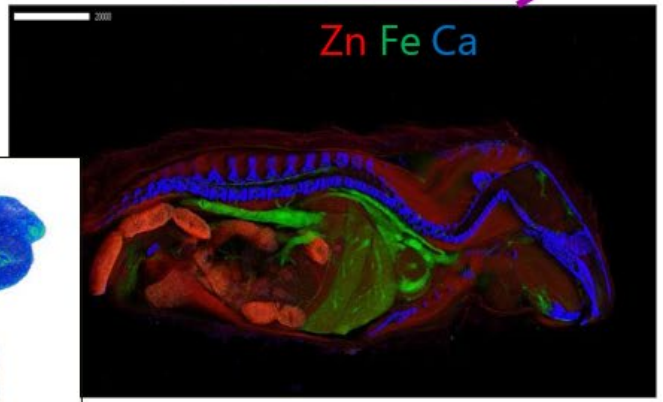
Subcellular level (nm) → Cellular level (µm) → Organ level (mm) → Organism level (cms)





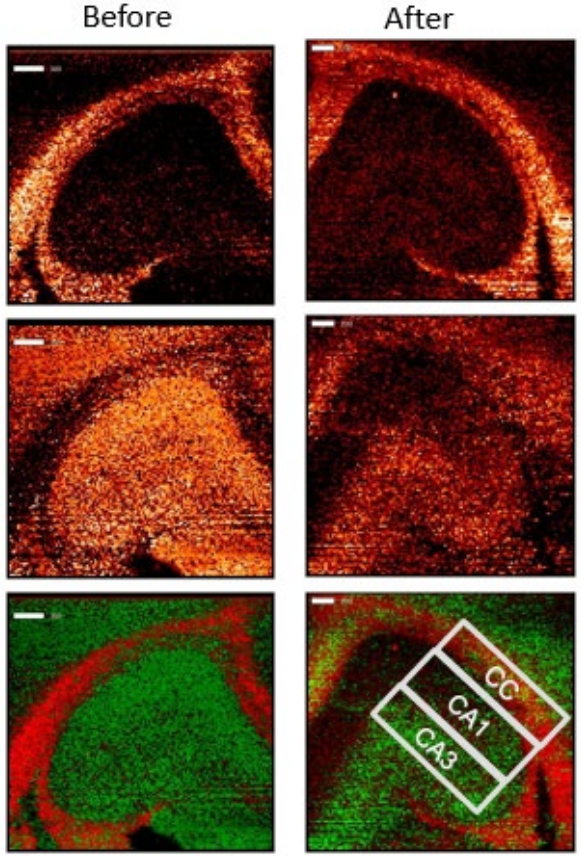




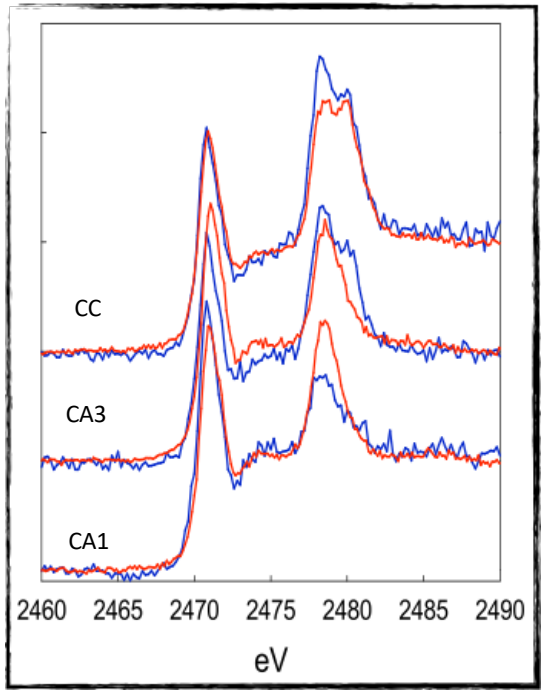


After B Hedman

ROLE OF SULFUR SPECIES IN STROKE



Sulfur K-edge spectra at specified locations

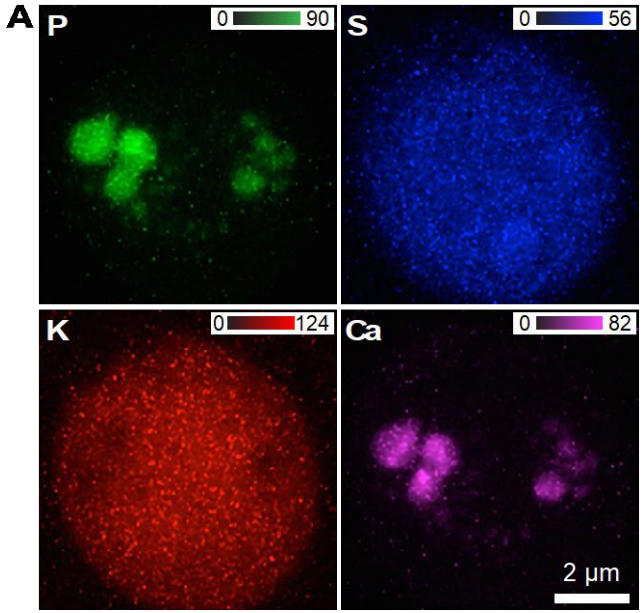


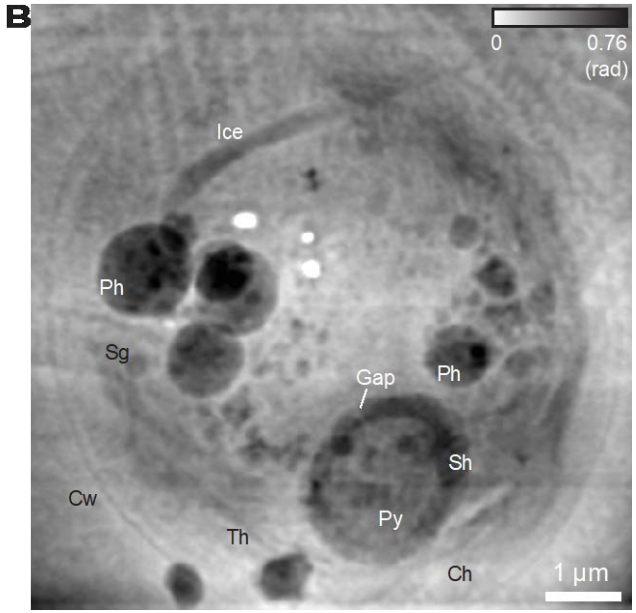
CA = pyramidal neurons and dendrites
CC = White Matter

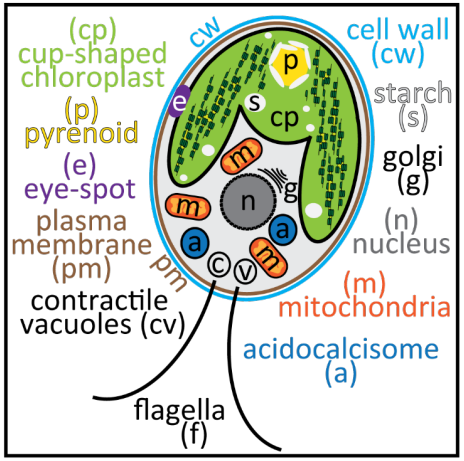
Change in concentration, speciation and location as a function of stroke

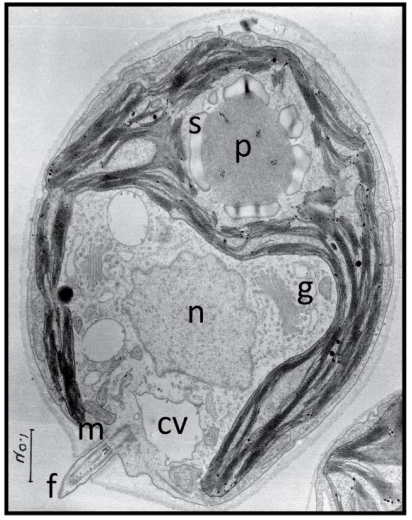
Work done at SSRL

CRYO-PTYCHOGRAPHY & XRF OF CHLAMYDOMONAS REINHARDTII







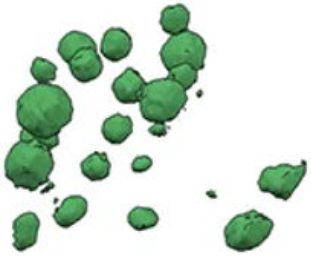


Junjing Deng et al., PNAS 2015

- 5.2keV, 70nm ZP, 167x151 Cartesian grid
- 0.5s exposure, 6.5h measurement
- white spots beam damage (not careful)
- ~20 nm resolution

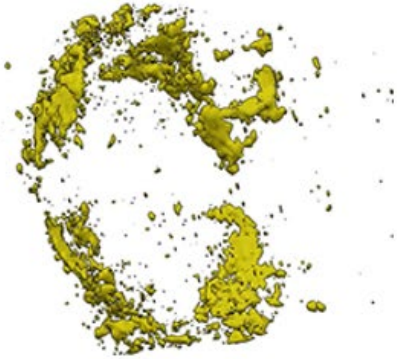
=> Beautiful structural visualization, strong contrast

Extended to 3D

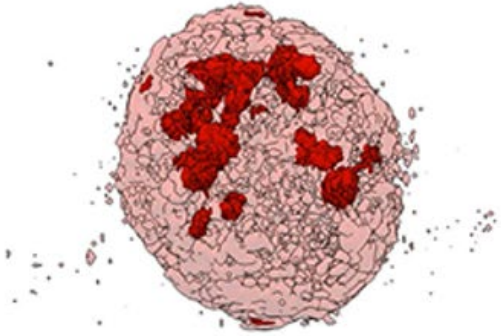


P

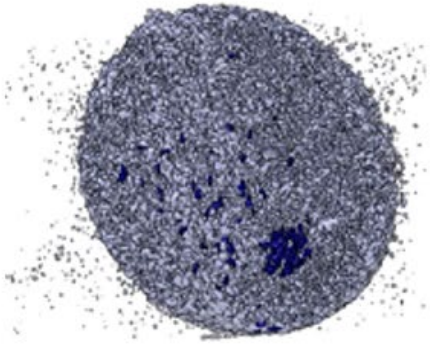
Ca



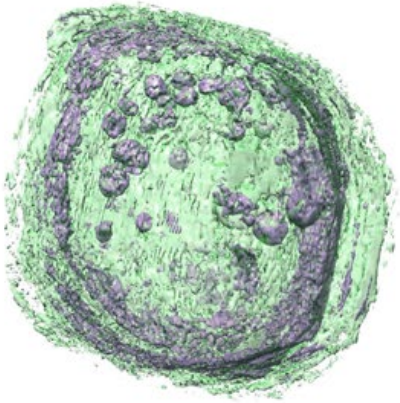
Cl



K



S



Ptycho

submitted

work done at APS

Courtesy Dr. Junjing Deng (APS)

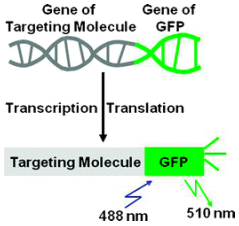
LBTS AS “GFP” FOR 3D NANOSCALE X-RAY IMAGING

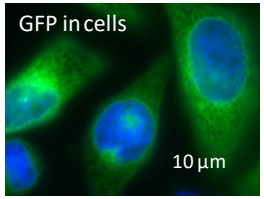
- GFP and YFP are ubiquitous for imaging individual proteins within cells and tissues with fluorescence microscopy
- Limitations to GFP tags:
  - large size: limits protein transport
  - use of visible light imaging can limits the spatial resolution of the technique

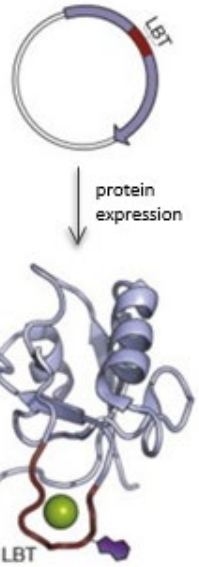
Lanthanide-Binding Tags (LBTs)

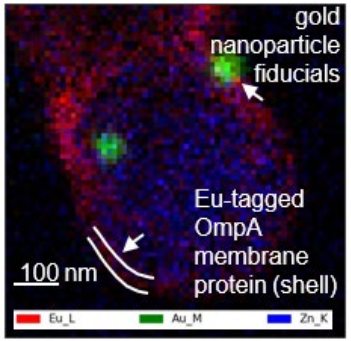
- Analogous application for nanoscale X-ray fluorescence microscopy
- LBTs are small peptides and easily fused to proteins and transported
- X-ray fluorescence microscopy improves the spatial resolution to ~10 nm in 3D

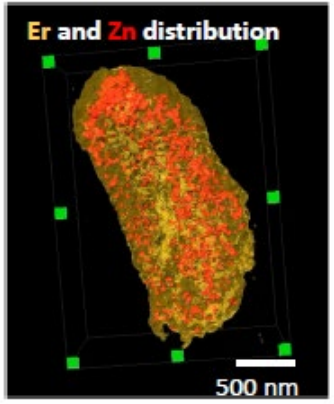
Lisa Miller (BNL), Karen Allen (Boston Univ), Barbara Imperiali (MIT)











Victor, et al., JACS 142:2145-2149 (2020).

Work performed at HXN beamline at NSLS-II and BNP beamline at APS

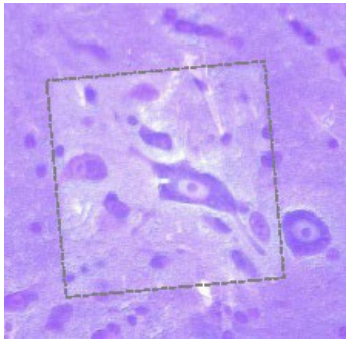
SAMPLE PREPARATION, SENSITIVITY, SPATIAL RESOLUTION AND RADIATION DAMAGE:

- Strongly depends on actual application. Higher resolution carries radiation damage implications
- mm sized samples, typically um resolutions
- 10s-100s of micron sized samples: sub um resolution, typically down to 20-30 nm
- For high resolution, high sensitivity (trace metals), thin, trace element-clean substrates
- X-ray microscopy, Limit is ~ 10^{10} Gy for frozen hydrated samples, ~10 nm structural resolution limit
- Discuss with beamline staff!

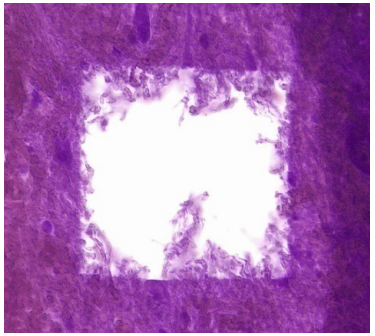
APS Today => minimum detectable Zn [#atoms]

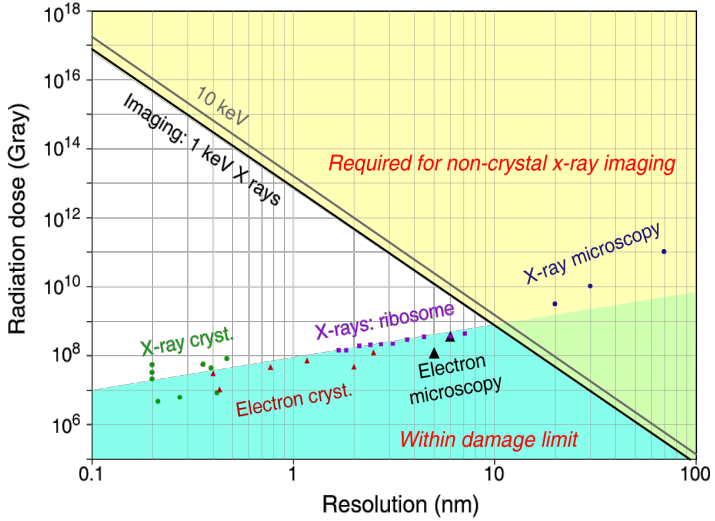
	Spot size	
sample thickness [um]	**200 [nm]**	20 [nm]
0.1 [um]	**3500**	35
10 [um]	**26000**	260

Fixed (p-formaldehyde), paraffin, scanned, rehydrated



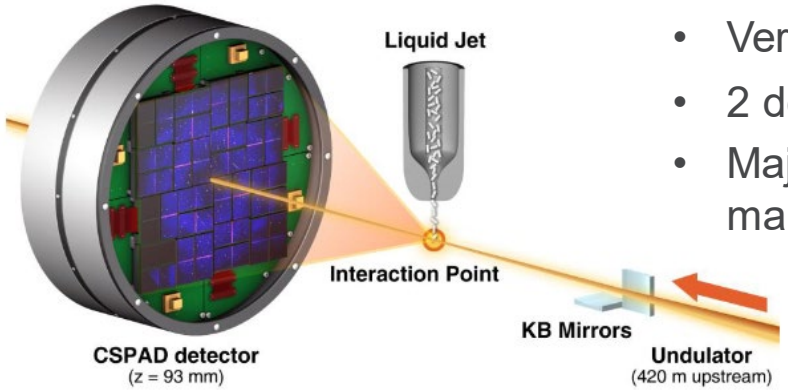
Freeze dried (unfixed), scanned, rehydrated





This plot: Howells *et al.*, *J. Electr. Spectr. Rel. Phen.* **170**, 4 (2009). See also Shen *et al.*, *J. Sync. Rad.* **11**, 432 (2004).

THE LCLS X-RAY LASER AT SLAC PROVIDES HIGH-RESOLUTION, DAMAGE-FREE, ROOM TEMPERATURE STRUCTURES AND DYNAMICS



- Very high brightness, short pulse X-ray source
- 2 dedicated instruments for structural biology
- Major upgrade underway (120 Hz to 1 MHz), marking a step-change in relevance to bioscience

High resolution structures

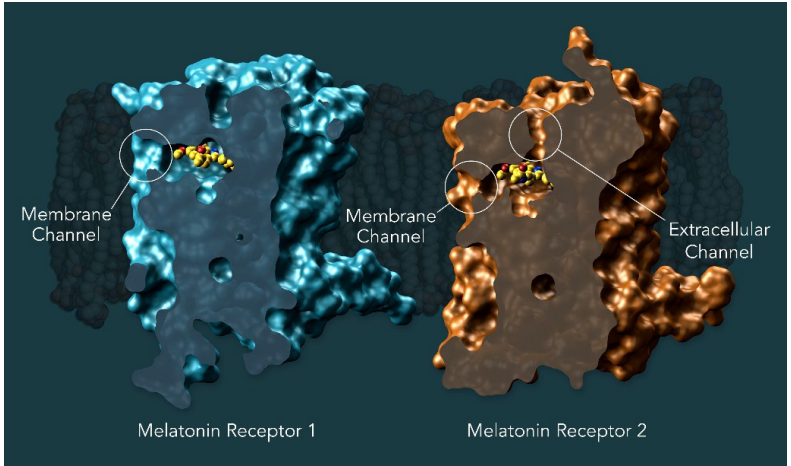
- Particularly suited to delicate and small (μm) crystals and low protein consumption (e.g. GPCRs)
- No crystal harvesting, and fast (days) optimization time
- Native-like membrane environment

Molecular dynamics

- Enzyme dynamics via “mix and inject” on **μs to ms** timescales
- Photo-excitation of proteins with chromophores on **ps to μs** timescale
- Structural dynamics (e.g. retinal, rhodopsin) on **sub-ps** timescale

IMAGING STRUCTURAL DYNAMICS WITH ULTRAFAST X-RAYS

- Radiation damage-free structural determination
  - High resolution metalloenzymes structure prior to photoreduction
  - Drug discovery: GPCRs in complex with ligands
- Structural dynamics at physiological conditions
  - Enzymatic reactions at physiological conditions
  - Antibiotic binding dynamics
  - Ligand binding to adenine riboswitch
- Multi-scale imaging in combination with cryo-EM
  - LCLS and cryo-EM combine to provide imaging from cells to molecules

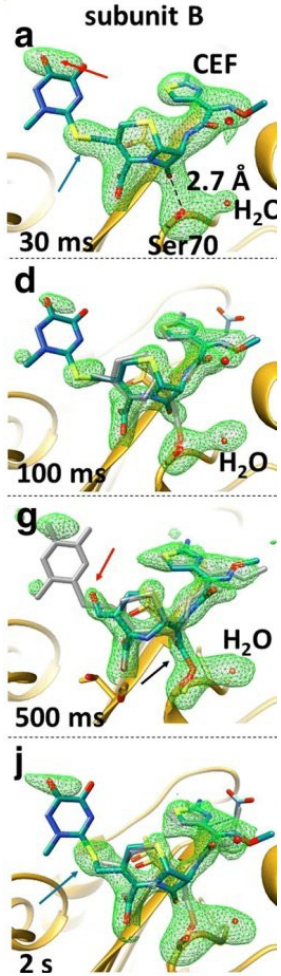


Melatonin receptors (MT1 & MT2) in complex with agonists and antagonists reveal receptor specificity

Stauch *et al.*, Nature **569**, 284-288, (2019)
Johansson *et al.*, Nature **569**, 289-292, (2019)

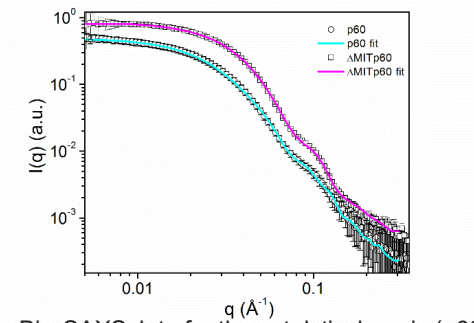
Time-resolved binding of third-generation antibiotic ceftriaxone to *Mycobacterium tuberculosis* β-lactamase

Olmos *et al.*, BMC Biology **16**, 59, (2018)



COMBINING SMALL ANGLE X-RAY SCATTERING AND CRYO-EM TO STUDY STRUCTURE OF KATANIN CATALYSIS SUBUNIT

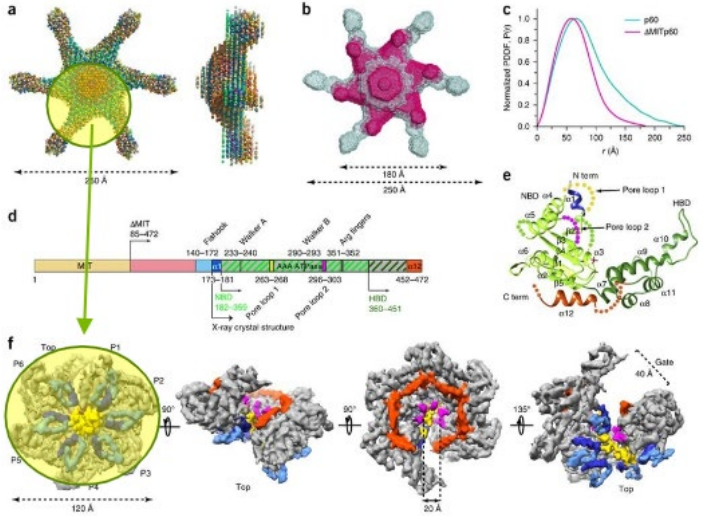
Cells constantly assemble and disassemble their microtubule cytoskeleton. Katanin is a microtubule-severing enzyme that generates internal breaks in microtubules, thus modulating their dynamics and organization. Owing to a lack of 3D structures, the mechanism of microtubule severing by this enzyme has remained poorly understood.



Bio-SAXS data for the catalytic domain (p60) of Katanin, and the core domain (ΔMITp60)

BioSAXS structure For full length

CryoEM structure For core only



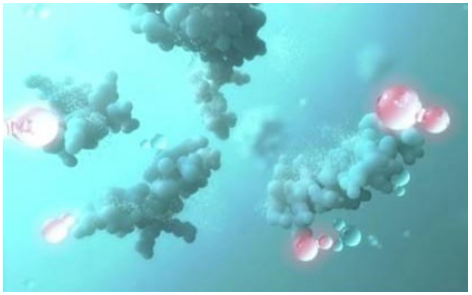
Flexible sequences are missed in crystal and cryo-EM structures, while they show in BioSAXS structures.

BioSAXS measurements performed at **12-ID-B**
Using Inline FPLC-SAXS and a home-designed temperature-controlled (~4C) flow cell

E. Zehr, A. Szyk, G. Piszczek, E. Szczesna, X. Zuo(APS) and A. Roll-Mecak(NIH), *Nat. Struct. Mol. Biol.* 2017, 24(9), 717-725

work done at APS

X-Ray Footprinting: a Solution State Method for Protein Structure



- Uses water locations to reveal the changes in protein conformation as a function of time or as a function of interactions
- Residue-specific resolution, both on protein surface and inside channels and cavities

Highlighted Publications

Overview of current instrumentation
Analytical Chem. 2020, 92, 1, 1565.

GPCR structure elucidation
Cell. 2019 May 16;177(5):1232.

Carotenoid protein structure and dynamics
J. Biol. Chem. 2019 294: 8848.

Protein-metal interactions
J. Am. Chem. Soc. 2017, 139 (36), 12647.

Where to perform the method

Currently two synchrotron beamlines in the United States are dedicated to X-ray footprinting:

Beamline 3.2.1 at the ALS
alsfootprint.snappages.site

Beamline 17-BM at the NSLS-II
case.edu/medicine/csb/

Link to short overview video

Supported by NIH-NIGMS

HOW CAN YOU MAKE USE OF THESE RESOURCES ?

- beamtime is available on most beamlines at most synchrotrons to outside users through a competitive proposal process.
- Proposal submission deadlines typically 2 or 3 times a year.
- Typically 80% or more of 'beamtime' on any beamline is distributed
- Some types of proposal:
  - General User Proposals
    - Open to anyone, just have to write a good proposal. Proposals get reviewed by committee, assigned based on scores. Proposals that don't quite make the score, 'age' so that they have a better chance next time.
    - Users typically come for experiments 3-4 days (9-12 shifts), carry out experiments with help of beamline scientist
    - No cost for beamtime, the expectation is that results will be published.
  - Proprietary Experiments
    - Are also possible, generally not published, but cost recovery of beamtime is required
- Most importantly: try to identify possible beamlines in advance, and **contact the beamline scientist** well **before writing the proposal.**

Feel free to contact me (svogt@anl.gov) (or point of contacts – later in slide), for help on general feasibility and potential beamlines/lightsources for a specific project.

SUMMARY

- Infrared Microscopy: chemical imaging of biological systems, based on differences in IR spectra (eg, lipids, proteins, protein folding, …). Resolution a few microns down and 10s of nm using near field methods.
- Transmission X-ray microscopy (TXM) (can be combined with tomographic approaches)
  - Soft X-ray range: typically to image cellular structure exploiting natural contrast between water and proteins, lipids, etc. resolution down to 30ish microns, < 10 um thickness.
  - Can be combined with spectroscopy (eg, STXM) for chemical imaging
  - Hard x-ray range: typically exploiting phase contrast resolution down to 20nm, thicker samples
  - Typically requires chemical fixation or cryogenic sample preservation
- Tomography / radiography – micron resolution, fast, can image live samples at reduced resolution. Phase contrast provides significantly increased contrast for biological (soft) samples

SUMMARY

- XAS Imaging / X-ray fluorescence microscopy / microspectroscopy
  - Macro to micro to nanoprobes, covering mm sized samples to 10s of micron sized samples, with resolutions from microns to 10s of nm. Typically fairly slow experiments.
  - Sensitivities down to ppm for trace element imaging using X-ray fluorescence (eg, P, …, Zn, ….)
  - Can combined with lensless imaging methods (eg, ptychography) to push structural resolution down to 10 nm
  - High resolution requires sample preservation (chemical fixation or cryo)
  - Can combine with spectroscopy to image chemical state (eg, Fe2+ vs Fe3+, …)
- Small Angle X-ray Scattering to ‘image’ protein shape
- Macromolecular Crystallography to ‘image’ protein structure
- X-ray Free Electron Laser (LCLS) to ‘image‘ ultrafast structural dynamics

- Combinations of these techniques as well as visible light microscopy and (cryo-) EM to address multimodal problems
- The future is bright: NSLS-II was just built, upgrades under way at LCLS and APS, planned for ALS, ...
  - Many of these techniques can expect gains of 2 orders of magnitude

https://als.lbl.gov/

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POC: Lisa Miller
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Also happy to discuss / direct any other question …

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THANK YOU !!!

https://www-ssrl.slac.stanford.edu/

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