

# **Biological Applications** of Cryogenic Detectors

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#### Outline

Cryogenic detectors characteristics:

- + High resolution
- + High broadband efficiency
- Small size
- Low speed



Three applications:

- X-ray absorption spectroscopy
- Time-of-flight mass spectrometry
- Fluorescence resonance energy transfer



### X-ray Absorption Spectroscopy (XAS)



Sample energy levels with natural-linewidth limited resolution



#### Fluorescence-Detected XAS with Cryodetectors



Cryogenic spectrometer : 9-channel STJ 10-20eV resolution, ~100,000 cts/s,  $\Omega/4\pi \approx 10^{-4}$ 

Cryogenic detectors enable the analysis of dilute samples whenever semiconductors lack energy resolution and gratings lack efficiency.



#### Why soft X-rays?



Absorption spectra on different Transitions ("edges") in  $NaMoO_4$ .

Low energy transitions are sharper and more sensitive to chemical states.



#### Photosynthetic Oxygen Production in PSII

Collaboration with V. Yachandra (Chem. Rev. 96, 2927 (1996)) and S.P. Cramer



Cryogenic spectrometers are sensitive to  $\sim 100$  ppm metal concentrations in proteins.

![](_page_6_Picture_0.jpeg)

#### Absorption spectra and Chemical State Analysis

![](_page_6_Figure_2.jpeg)

Absorption fine structure is sensitive to oxidation state, ligand field and bond symmetry.

![](_page_7_Picture_0.jpeg)

#### ACDS: Acetyl Metabolism in Methanogens

Collaboration with D. Grahame (USUHS) and S.P. Cramer (UCD)

Acetyl Coenzyme-A Decarbonylase Synthase catalyzes the reversible formation of acetyl from CO and  $CH_3$ 

![](_page_7_Figure_4.jpeg)

![](_page_7_Picture_5.jpeg)

Previously proposed structure and reaction mechanism of NiFe cluster in  $\beta$  subunit of ACDS

![](_page_8_Picture_0.jpeg)

#### Structure of the active site in the ACDS $\beta$ subunit

![](_page_8_Figure_2.jpeg)

![](_page_8_Figure_3.jpeg)

![](_page_9_Picture_0.jpeg)

#### Chemical States of Ni in ACDS Active Site

![](_page_9_Figure_2.jpeg)

Acetyl catalysis occurs at two dissimilar Ni sites.

![](_page_10_Picture_0.jpeg)

### Outlook: Bioscience at the Synchrotron

Detector challenges for absorption spectroscopy:

1) Size and sample-detector distance

2) Everything else (quantum efficiency, speed, resolution, energy range,...)

Metals in biology (V, Mo, N, e.g. nitrogenase) and environmental science (Cr, As, Se...)

Possible future experiments: X-ray Spectro-microscopy 50 nm resolution, full-field imaging, 3D tomography

![](_page_10_Figure_7.jpeg)

![](_page_11_Picture_0.jpeg)

#### Time-of-Flight Mass Spectrometry (TOF-MS)

![](_page_11_Figure_2.jpeg)

MALDI and electrospray ionization allow 'soft' launch of large proteins.

Conventional microchannel plate detectors lose efficiency for large masses.

Cryogenic detectors 1) increase detection sensitivity for large masses, and 2) can discriminate between different ion charge states.

![](_page_12_Picture_0.jpeg)

### Commercialization of Cryogenic Mass Spectrometer

![](_page_12_Figure_2.jpeg)

Comet AG, Flamatt, Switzerland; www.comet.ch

Applications:

Protein complex research: Identification of large proteins and fragments Virus detection: Medical diagnostics, national security (LLNL) Genetics: DNA/ RNA genotyping (UNe), recognition of mutations

![](_page_13_Picture_0.jpeg)

#### Fluorescence Resonance Energy Transfer (FRET)

![](_page_13_Figure_2.jpeg)

FRET measures interatomic distance (changes) between fluorescence markers.

![](_page_14_Picture_0.jpeg)

#### FRET: Single-molecule detection

#### Confocal Microscopy

![](_page_14_Figure_3.jpeg)

2D and 3D imaging is possible.

![](_page_14_Picture_5.jpeg)

Single molecule detection requires:

- Small excitation volume (low background)
- Elimination of background fluorescence Confocal microscopy
- High efficiency collection and detection Interference filters
- Low dark count detectors Avalanche photodiodes

Confocal microscopy enables FRET spectroscopy on single molecules

![](_page_15_Picture_0.jpeg)

#### FRET with Cryogenic Detectors

Fluorophores exist that change emission wavelength with ambient conditions (pH, ion concentration)

One can then measure environmentally driven structural changes (time-resolved).

![](_page_15_Figure_4.jpeg)

Cryogenic detectors enable multi-wavelength single molecule spectroscopy.

Challenges:

- Better phosphors (wider wavelength range, lower intrinsic yield fluctuations, less photo bleaching) and better labeling (site specific, easy to introduce) important.
- Does increased capability justify large investment in cryogenic equipment?

![](_page_16_Picture_0.jpeg)

## Summary/ Outlook

![](_page_16_Picture_2.jpeg)

Fluorescence-detected X-ray absorption spectroscopy at the synchrotron is used to measure chemical states of dilute samples.

Cryogenic detectors enable soft XAS where semiconductor detectors lack resolution and gratings lack efficiency.

They are used to examine reaction mechanisms in metalloproteins. Larger area detectors are needed for higher sensitivity.

![](_page_16_Figure_6.jpeg)

![](_page_16_Picture_7.jpeg)

Future biological applications may include:

- X-ray spectro-tomography to relate chemical to structural information.
- Multi-wavelength single molecule spectroscopy to understand environmentally driven conformational changes of proteins.