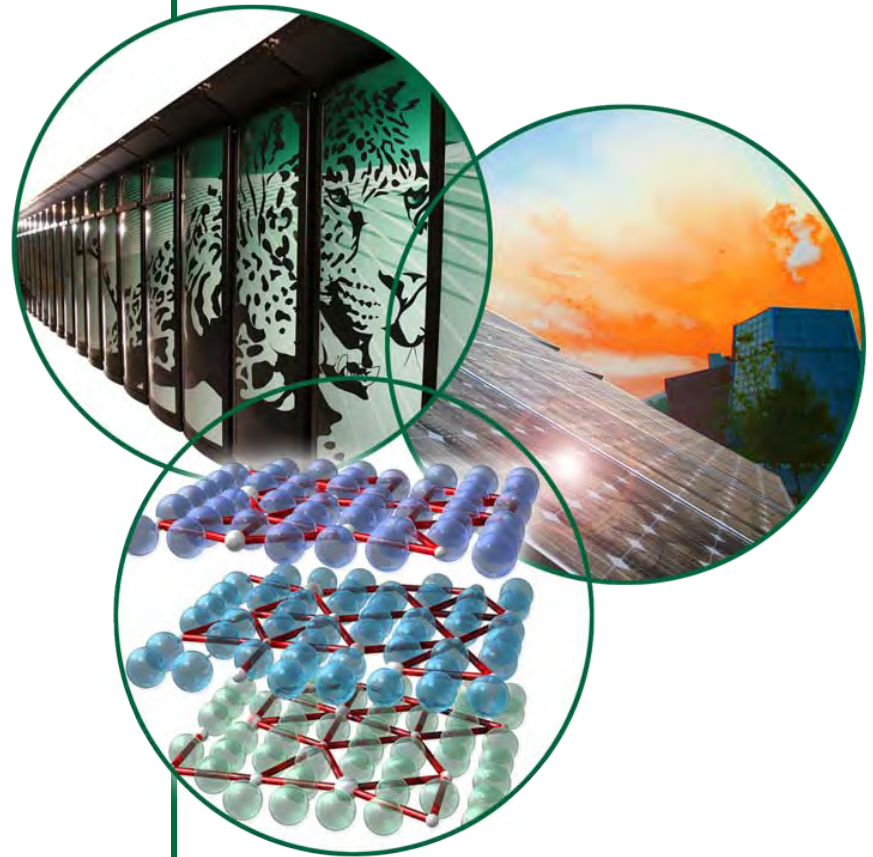


Neutron Scattering in Life Sciences

Dean Myles

Neutron Scattering Science
Division

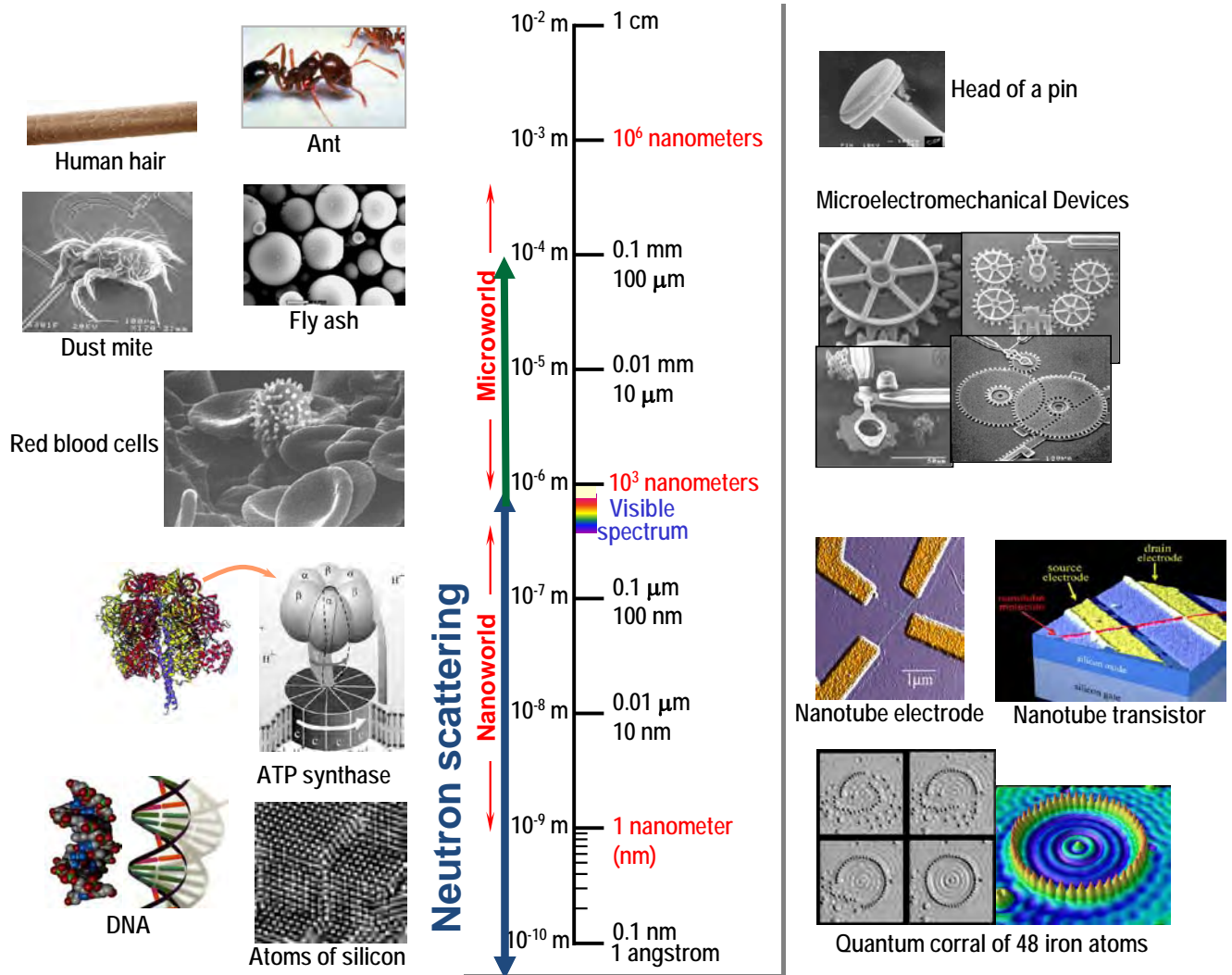


Excellence in Science

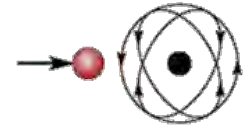
- Carry out world class research
- Provide an outstanding User Science Program
- Develop cross cutting signature science programs and partnerships
- Stay at the leading edge of neutron science by developing new capabilities, instruments, and tools.



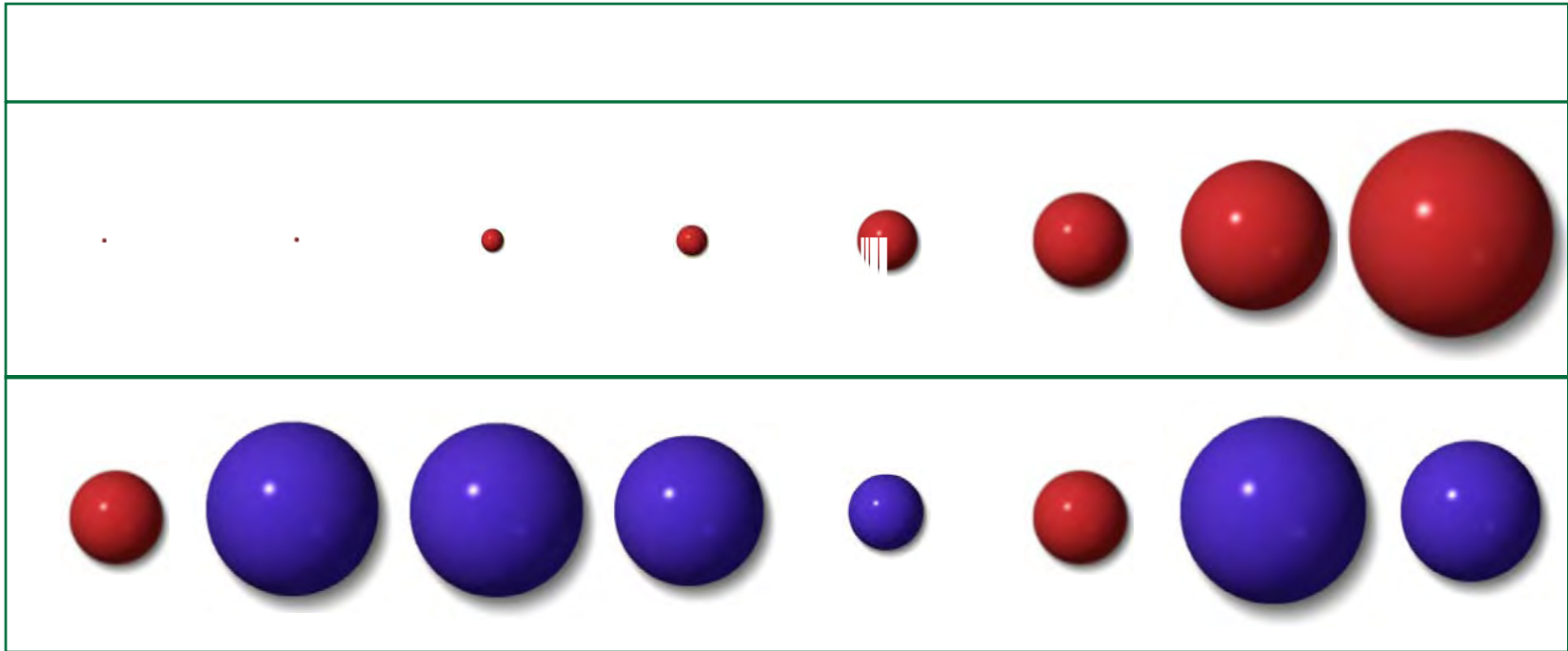
Neutrons – Angstroms to microns



Neutrons and Biology



Neutrons see the Nuclei....



- X-rays interact with *electron clouds* of atoms
- Neutrons interact with *nuclei*: better spatial resolution
- Large difference in the cross-section among isotopes
- Magnetic moment

Neutrons and Biology:

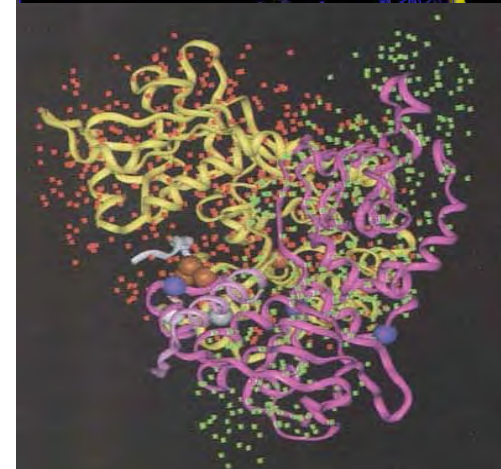
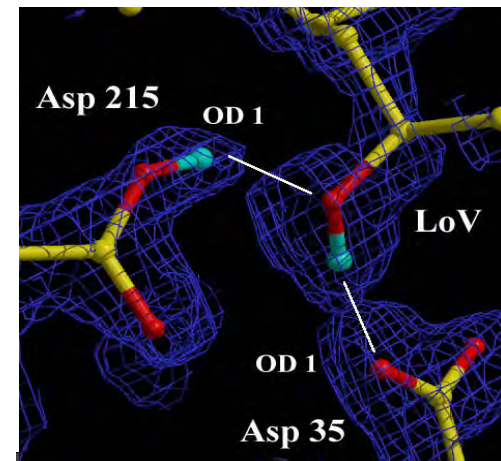
ORNL will provide world-leading instruments for neutron scattering at HFIR and at SNS

Neutrons are excellent probes for Hydrogen – and can discriminate between hydrogen and deuterium

Function: **H/D** in enzyme mechanism;
proton shuttling & transfer

Structure: **H/D Labeled protein** in
complex systems

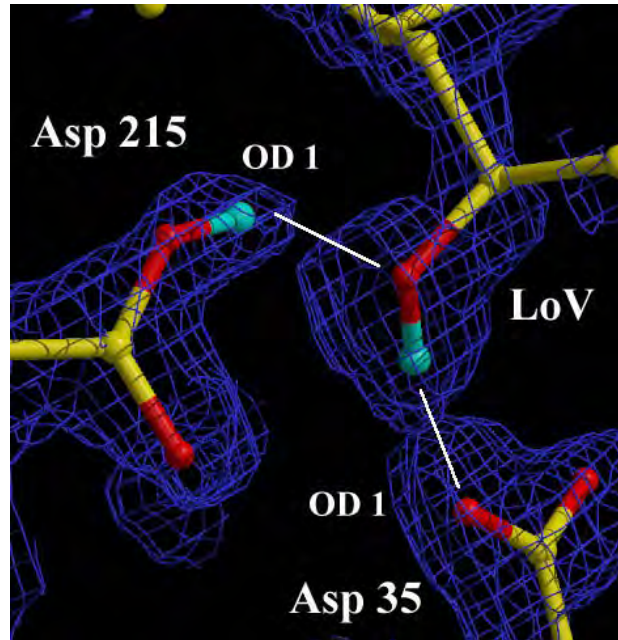
Dynamics: Specific **H-Labeling** in
deuterated systems



Neutron Macromolecular Crystallography

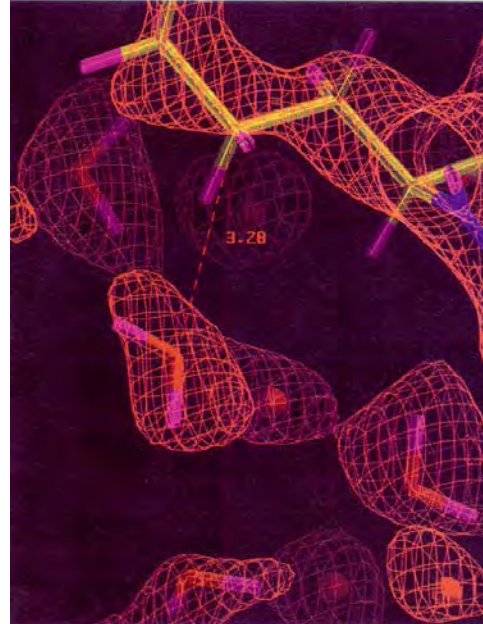
Hydrogen atoms: Invisible agents of Biological Activity

Resolution 2.1Å



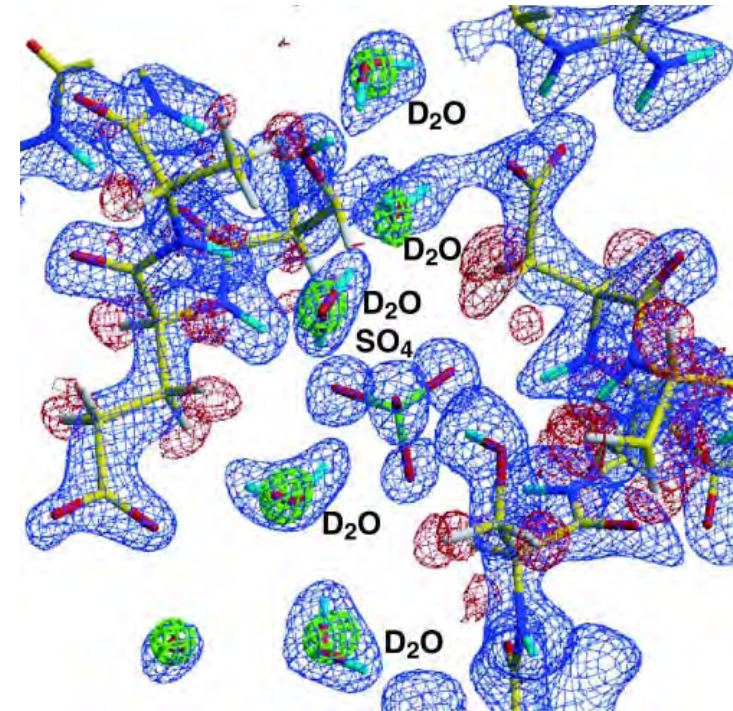
Coates *et al.*, *Biochem*, (2001)
40(44):13149-57

Resolution 1.7Å



Bon *et al.*, *Acta Cryst* (1999)
D55:978-87

Resolution 1.5Å



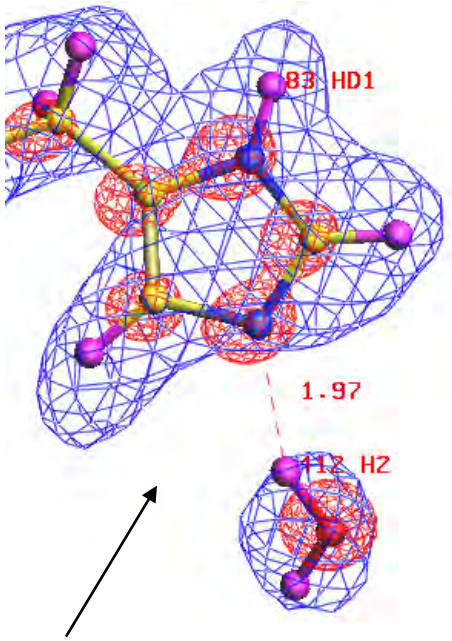
Chatake *et al.*, *Proteins* (2003) 50:516-23

NMX is a unique experimental tool for the determination of key hydrogen atom and water molecule positions in biological macromolecules (enzymatic mechanism, substrate binding, solvent analysis...)

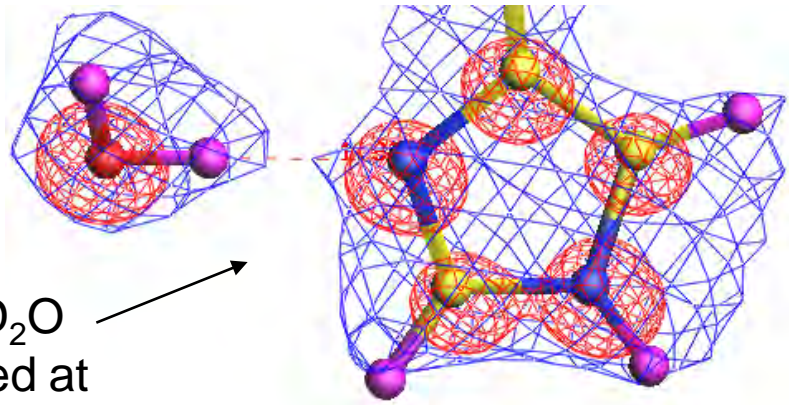
Protonation State and Catalytic Mechanism in Human Aldose Reductase

Blakeley et al. PNAS, 105, 1844, 2008

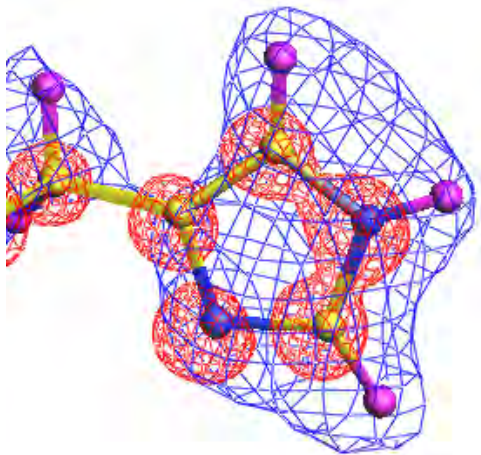
$2F_o - F_c$ positive neutron maps in blue
 $2F_o - F_c$ X-ray electron density maps in red.



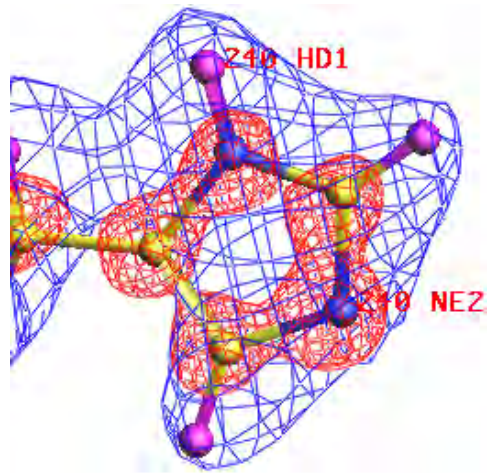
Histidine 83 + D₂O
(protonated at ND1)



Histidine 110 + D₂O
(Singly protonated at NE2)



Histidine 187
(protonated at NE2)

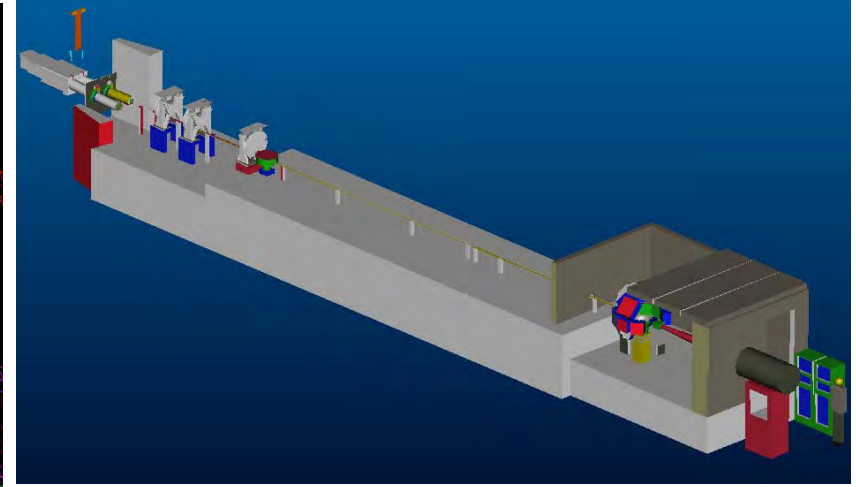
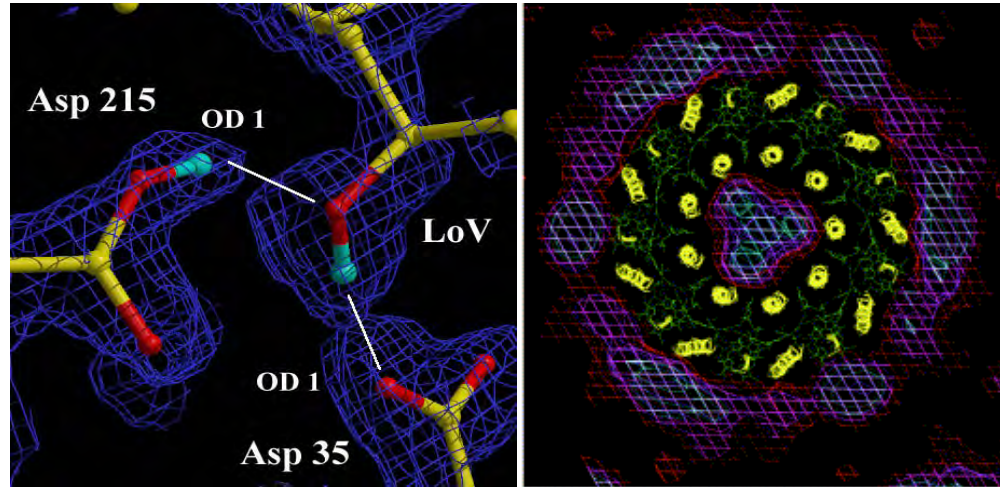


Histidine 240
(protonated at ND1)

MaNDi – Macromolecular Crystallography at SNS

IDT: A.Mesecar (Chicago), P. Thiyagarajan, A.J. Schultz (IPNS, Argonne), P.Langan (LANL), D. Myles (ORNL)

Hydrogen atoms: Invisible agents of Biological Activity



High resolution (1.5-2.0Å)

Enzyme mechanisms
Ligand binding interactions
Solvent structure
H/D exchange

Low resolution (>10Å)

Lipid & detergents
Membrane proteins

- Optimized for larger proteins and complexes
- High data rates (10 to 50X of existing facilities) and high resolution
- 1 mm³ crystals with lattice repeat **up to 150 Å** and $d_{min} = 2.0 \text{ Å}$
- 0.125 mm³ crystals of deuterated proteins

Bio-SANS at HFIR – Dedicated to Biology

First data from the HFIR Bio-SANS instrument

User Program launched 2007

Applications

Bio-macromolecules and assemblies•

Protein complexes

Protein/DNA complexes

Lipids

Viruses

Hierarchical biological structures•

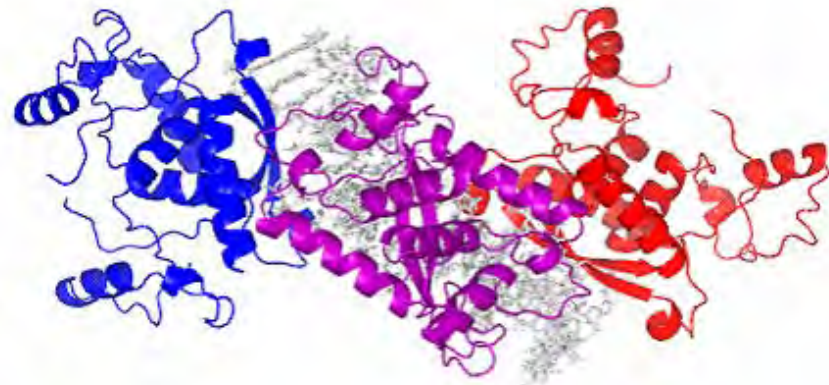
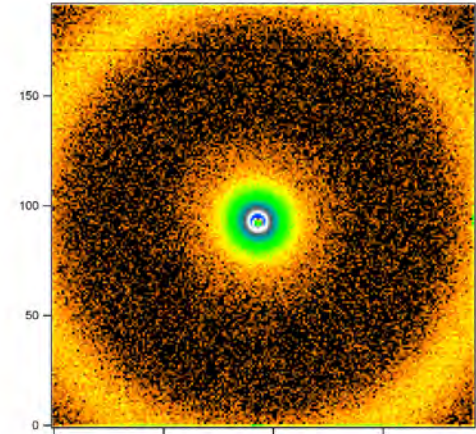
Gels

Fibers and fibrils

Vesicles/emulsions

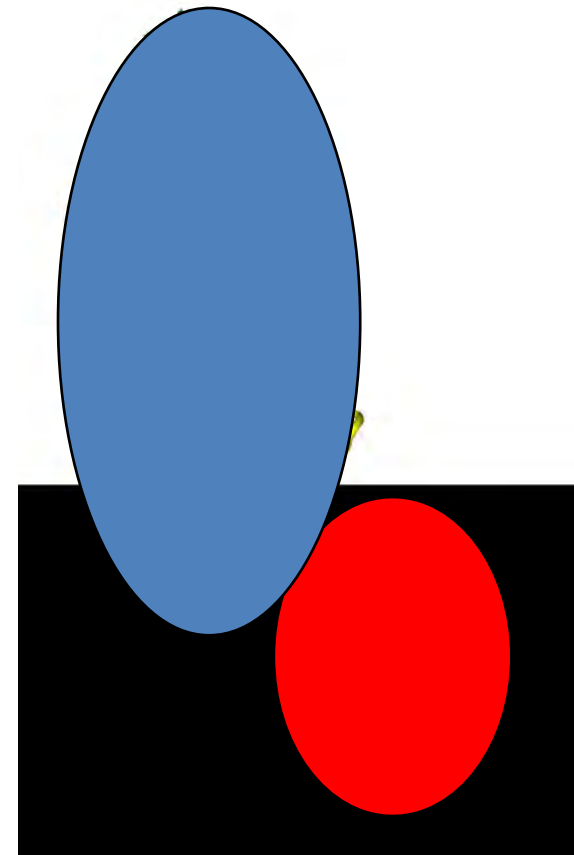
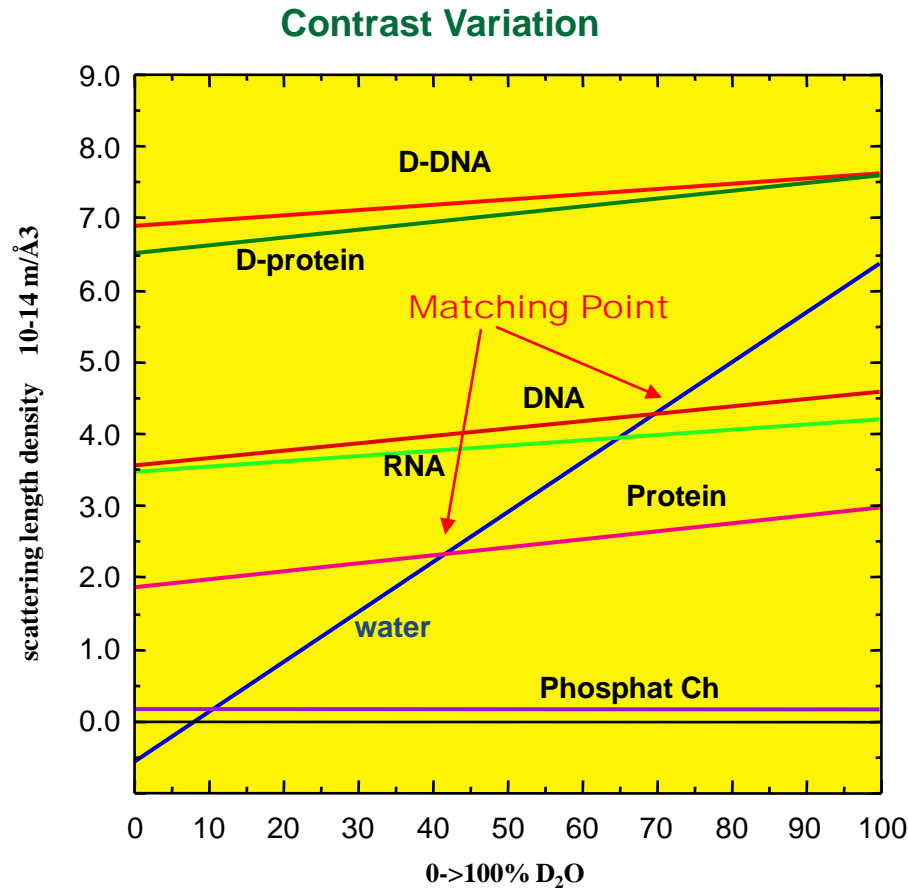
Membrane diffraction

Bio-mimetic systems



Small-angle Solution Scattering with Computational Modeling Methods

Assembling protein components into functional units



Solution Studies of Protein Complexes

Assembling protein components into functional units

Low-Resolution Techniques provide molecular envelope shapes

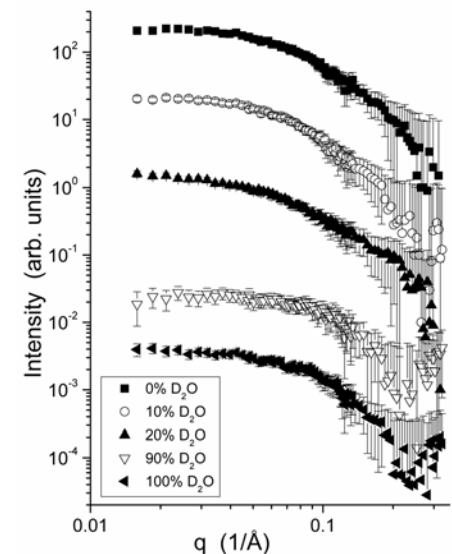
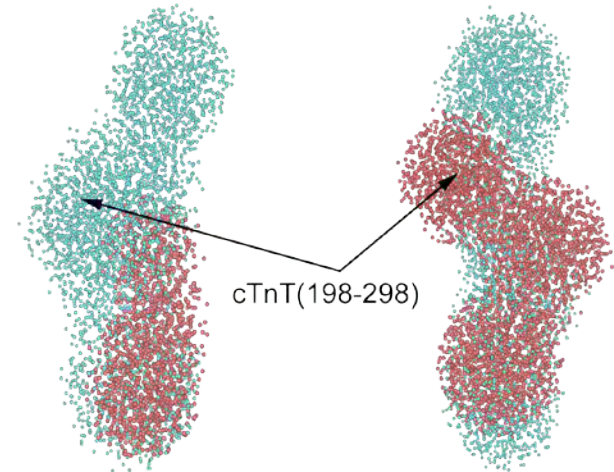
➤ Small-angle Solution Scattering with Computational Modeling Methods

solution-state

useful range 10-1000Å (~15 – 500 kDa)

Visualizing interactions:

- 15-30Å maps of complexes to identify relative positions of the individual components (interaction surfaces)
- reconstruction of the high-resolution image from the X-ray structures of the individual components

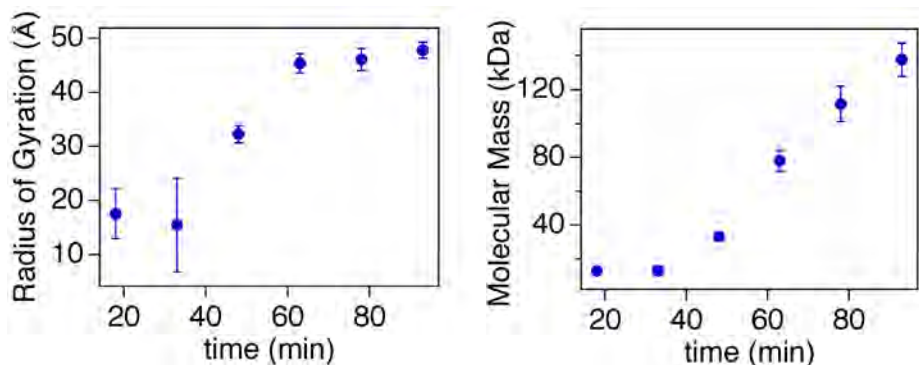


Understanding Polyglutamine Aggregation in Huntington's Disease

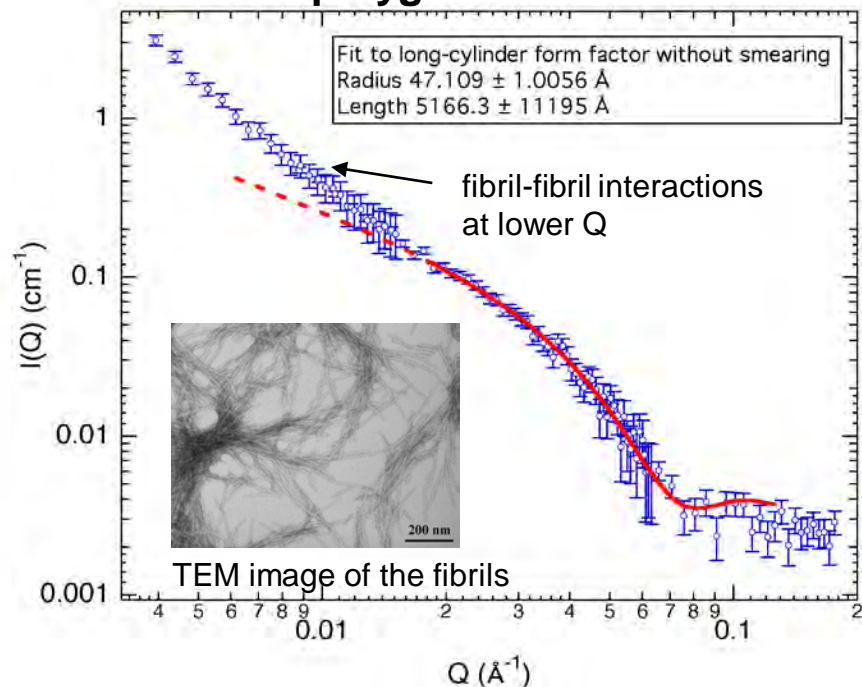
Christopher Stanley (ORNL) and Valerie Berthelier (UT Medical Center)

Huntington's disease is caused by an abnormal polyglutamine expansion in the huntingtin protein.

Using time-resolved small-angle neutron scattering (SANS), we capture "snapshots" of the protein as it aggregates:



SANS on polyglutamine fibrils:



With SANS, we are learning about the structural formation of the aggregates related to Huntington's disease. This work will aid us in determining the toxic mechanism and developing treatments for the disease.

SANS experiments were performed on the BioSANS instrument at the High Flux Isotope Reactor (HFIR), ORNL.

SANS Study of Switch Grass Breakdown

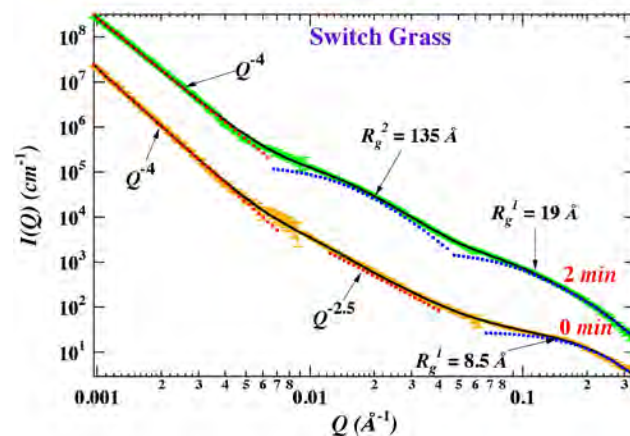
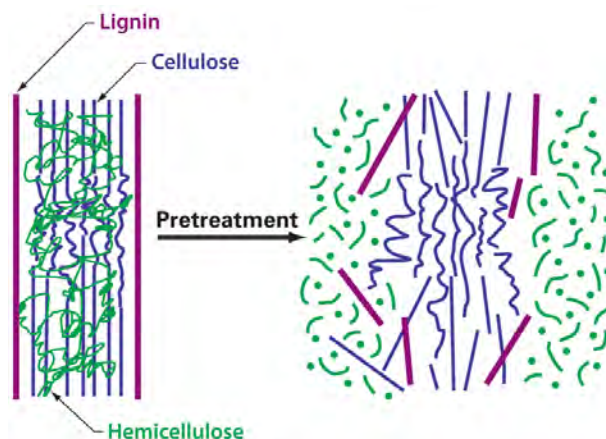
Sai Venkatesh Pingali, Volker Urban, Hugh O'Neill, Joseph McGaughey, Barbara Evans, William Heller, Dean Myles



Structural Studies of Biomass Degradation for Cellulosic Ethanol Production using CG-3 BioSANS



Switchgrass. Photo Courtesy: [unintelligible]

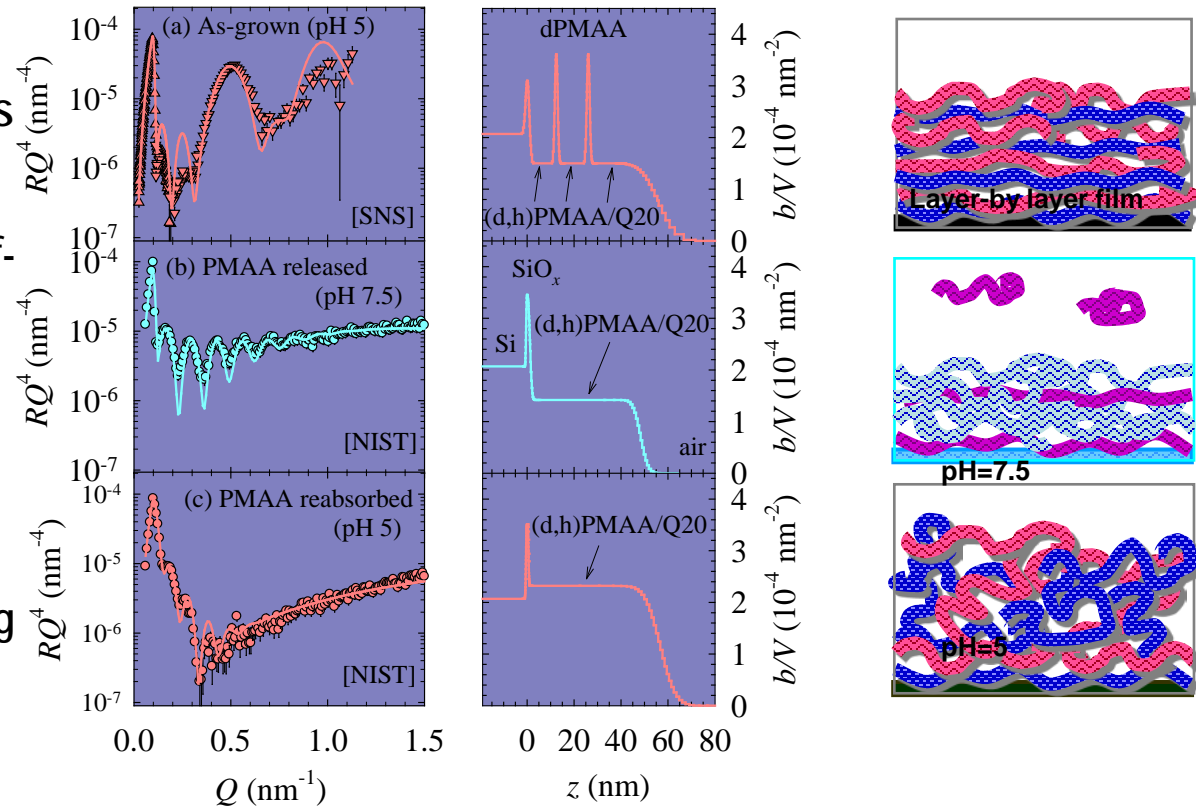


Structural changes in switch grass over pretreatment time:

- Primary subunit swelled from $\sim 8.5 \text{ \AA}$ (0 min) to $\sim 19 \text{ \AA}$ (2 min).
- In the initial state (0 min), cellulose fibrils form a random network ($Q^{-2.5}$).
- On treatment (2 min), large particles $\sim 135 \text{ \AA}$ appear possibly Lignin lumps.
- Micron-sized superstructures with smooth interfaces (roughness $< 50 \text{ nm}$) persist even after 10 min pretreatment (Q^{-4}).

Liquids Reflectometer: Study of Thin Organic Films and Interfaces

- Weakly coupled polyelectrolyte multilayers (WPEMs)- a new class of nanostructured materials
- Obtained through alternating self-assembly of water-soluble polymers carrying positive and negative charges
- Responsive polymers
 - bind and release dyes or drugs
 - fabricate novel metal-containing inorganic nanocomposite materials
 - control electro-osmotic flow in microchannels
- Neutron reflectometry- a powerful tool for understanding the structure and mechanism of self assembly



The layer-by-layer deposited film shows multilayer structure with NR. When the pH is adjusted one film component leaves. After lowering the pH, the film reassembles.

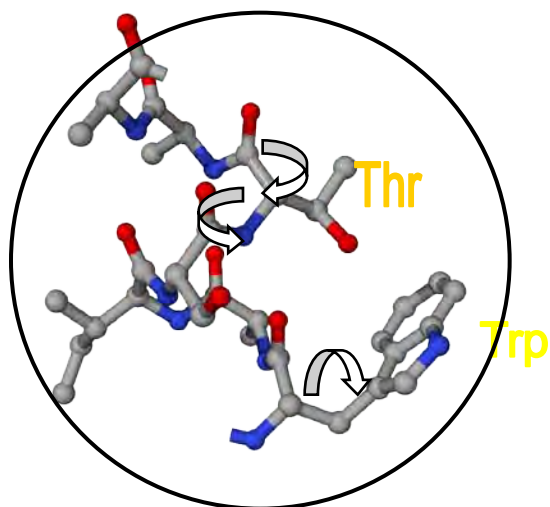
[1] Eugenia Kharlampieva, John F. Ankner, Michael Rubinstein, and Svetlana A. Sukhishvili, *Phys. Rev. Lett.* **100**, 128303 (2008).

[2] Eugenia Kharlampieva, Veronika Kozlovskaya, John F. Ankner and Svetlana A. Sukhishvili, *Langmuir*, **24**(20), 11346 (2008).

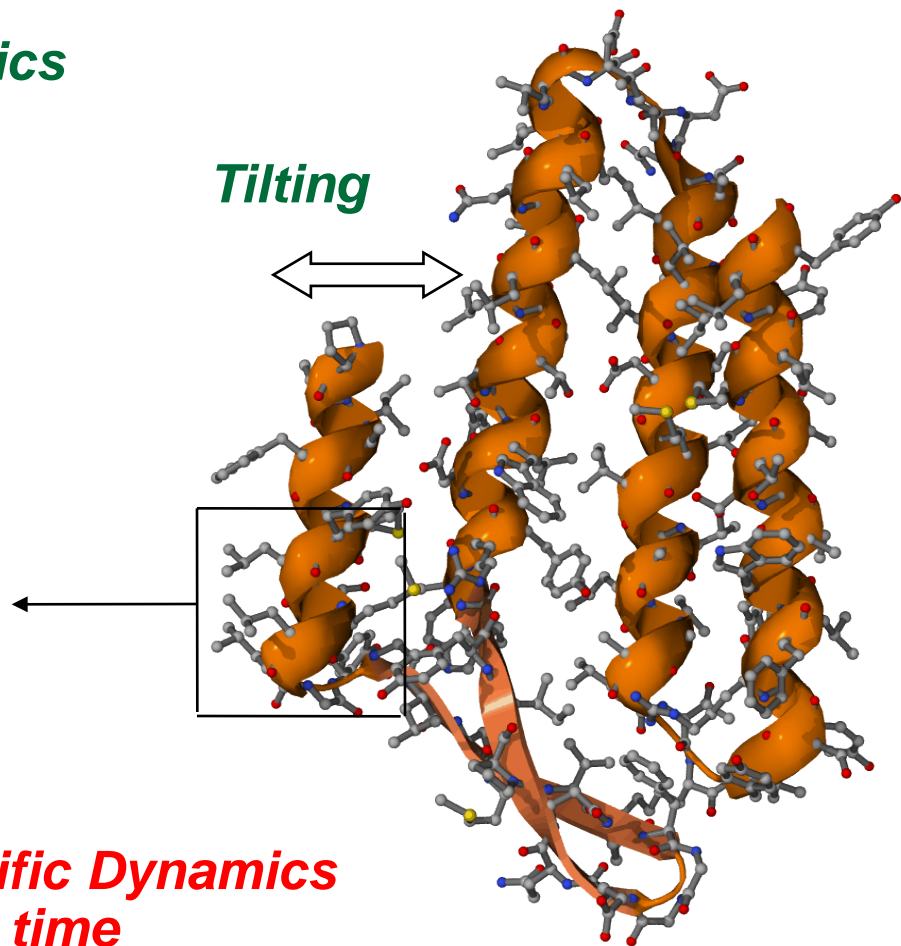
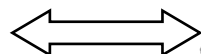
Neutron Spectroscopy provides Dynamics too!

Global Molecule Dynamics

Internal Dynamics



Tilting



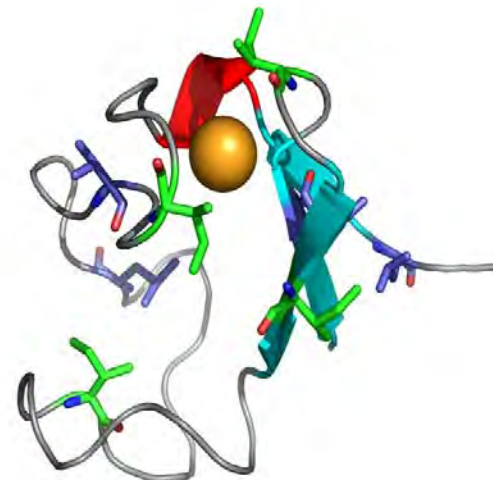
Translation

**H-labeling - Residue Specific Dynamics
- one residue at a time**

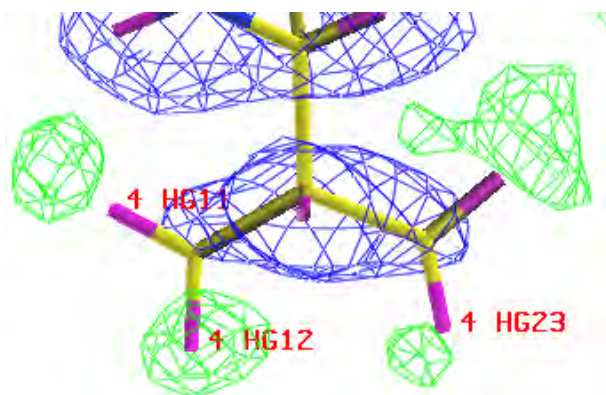
Rotation

H/D- Labelling: Correlating Protein Structure with Group, Residue and Regio-Specific Dynamics

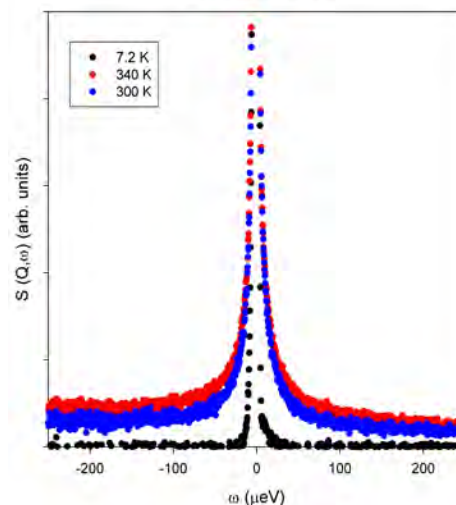
	C	N	O	H	D
Bcoh (fm)	+6.6 5	+9.36	+5.81	-3.74	+6.67
σ_{inc} (barns)	0	0.49	0	80.27	2.05



Structure - Diffraction



Initial BASIS data
hydration = 0.38



Molecular Dynamics:
Slowest mode of
Rubredoxin at 10 ns
time-scale with Leu/Ile
high-lighted

Incoherent scattering: Dynamics - QENS

The ORNL Center for Structural Molecular Biology

An Integrated Platform for Structural Biology

Bio-SANS at HFIR

- Macromolecular complexes

Membrane Diffraction

- Bio-mimetic membranes & systems

Neutron Protein Crystallography

- Protein structures at atomic resolution

Bio-Deuteration Laboratory

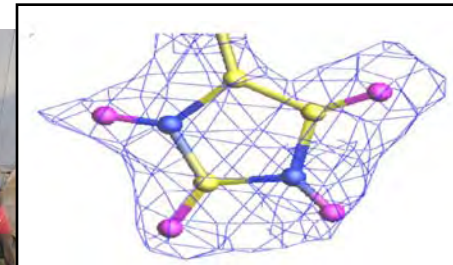
- H/D-labeled proteins & molecules

Computational Methods

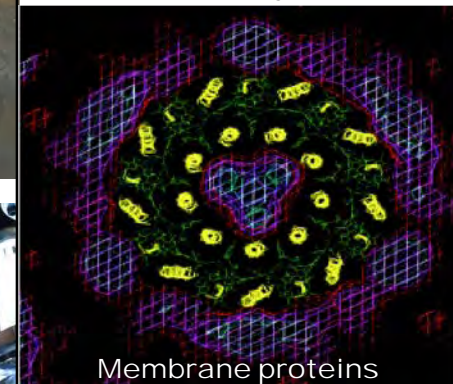
- Modeling protein complexes

Supporting Instrumentation

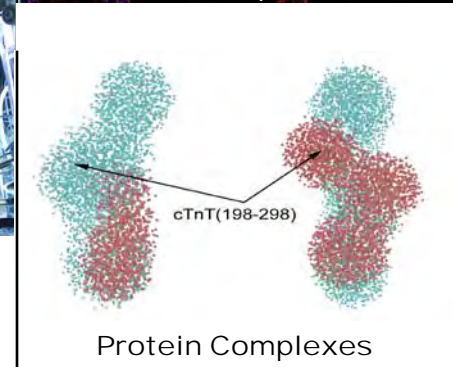
- X-ray crystallography, SAXS, light scattering, spectroscopy



Bio- catalysis



Membrane proteins



Protein Complexes

Delivering Science

Operational
Before 2008

Operational
During 2008

2009

2010-2011

2012-2014

TBD

1B – NOMAD - Disordered
Mat'ls Diffractometer

18 – ARCS - Wide Angle
Chopper Spectrometer

17 – SEQUOIA - High
Resolution Chopper Spect

2 - Backscattering
Spectrometer

1A– USANS - Ultra-
SANS Instrument

16b – VISION –
Vibrational Spectrometer

BL 16a

3 – SNAP - High
Pressure Diffractometer

15 – NSE – Neutron Spin Echo

14B – HYSPEC -
Hybrid Spectrometer

BL 14A

13 – FNPB -
Fundamental
Physics
Beamline

4A - Magnetism
Reflectometer

4B - Liquids
Reflectometer

5 – CNCS - Cold Neutron
Chopper Spectrometer

12 – TOPAZ - Single
Crystal Diffractometer

11B – MANDI -
Macromolecular
Diffractometer

BL 8A/B

9 – CORELLI - Elastic Diffuse
Scattering Spectrometer

6 – EQ-SANS

BL 10

11A – POWGEN3 -
Powder Diffractometer

7 – VULCAN - Engineering
Diffractometer

Neutrons in Structural Biology

Conference on New Frontiers in Neutron Macromolecular Crystallography

- George Detittia
- Jenny Glusker
- Herbert A. Hauptman
- Wayne Hendrickson
- Andrzej Joachimiak
- Anthony Kossiakoff
- Brian Mathews
- Alberto Podjarny
- Dagmar Ringe
- Gerald Stubbs
- B.C. Wang

+ Andrew Mesecar and the >60 members of the MaNDi IDT



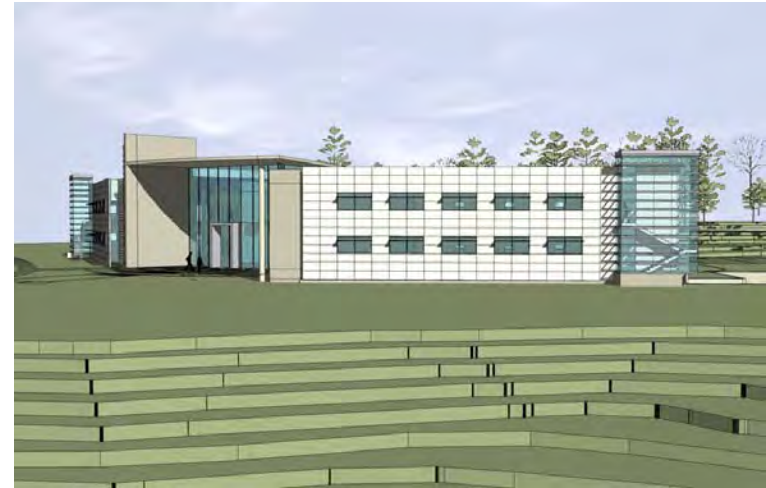
If You Build it, "We" Will Come

Opportunities to Engage and Partner

- Access is FREE – Peer reviewed proposals
 - 75% in open access – deadline is June, 2009
- Join one of the new Instrument Development Teams
 - IMAGINE Structure and Function
 - Cold neutron triple-axis Dynamics
 - SERGIS Spin echo encoding of scattering angle
 - VENUS Imaging and Tomography
- Form a team to develop instrumentation that will complement the current instrument suite
- Partnerships for Science

UT-ORNL Joint Institute for Neutron Sciences (JINS)

- Created as a part of agreement for UT-Battelle to run ORNL.
- JICS (Computer Science), JIBS (Biological Science), and JIAM (Advanced Materials)
- Mission and Goals
 - Collaboration/research grants
 - Neutron Fellowships
 - Neutron seminars:
 - Sabbaticals
 - Workshops and schools



Join us at the world's foremost center for the study of materials using neutrons

- SNS and HFIR provide unprecedented capabilities for understanding the structure and properties of materials across the spectrum of biology, chemistry, physics, and engineering
- Stay at the leading edge of neutron science by developing new capabilities, instruments, and tools.

