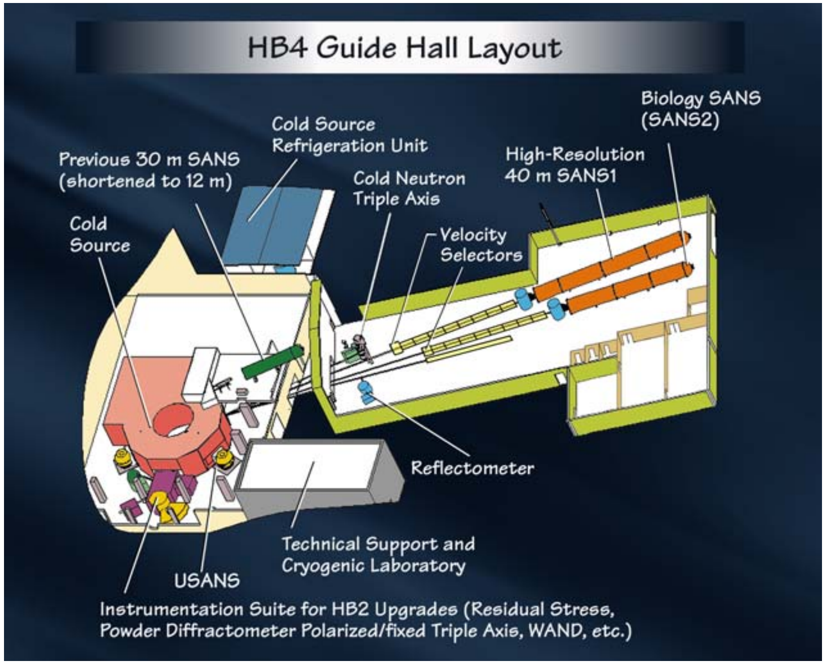
Neutron Scattering in Biomedicine & Bio-Materials

Dean Myles
Center for Structural Molecular Biology
Oak Ridge National Laboratory
Oak Ridge, Tennessee

The Centre for Structural Molecular Biology

An OBER funded resource for the biological community
Specialized tools for analysis of biomolecular complexes
D-Lab, Bio-SANS, Reflectometry, MaNDi

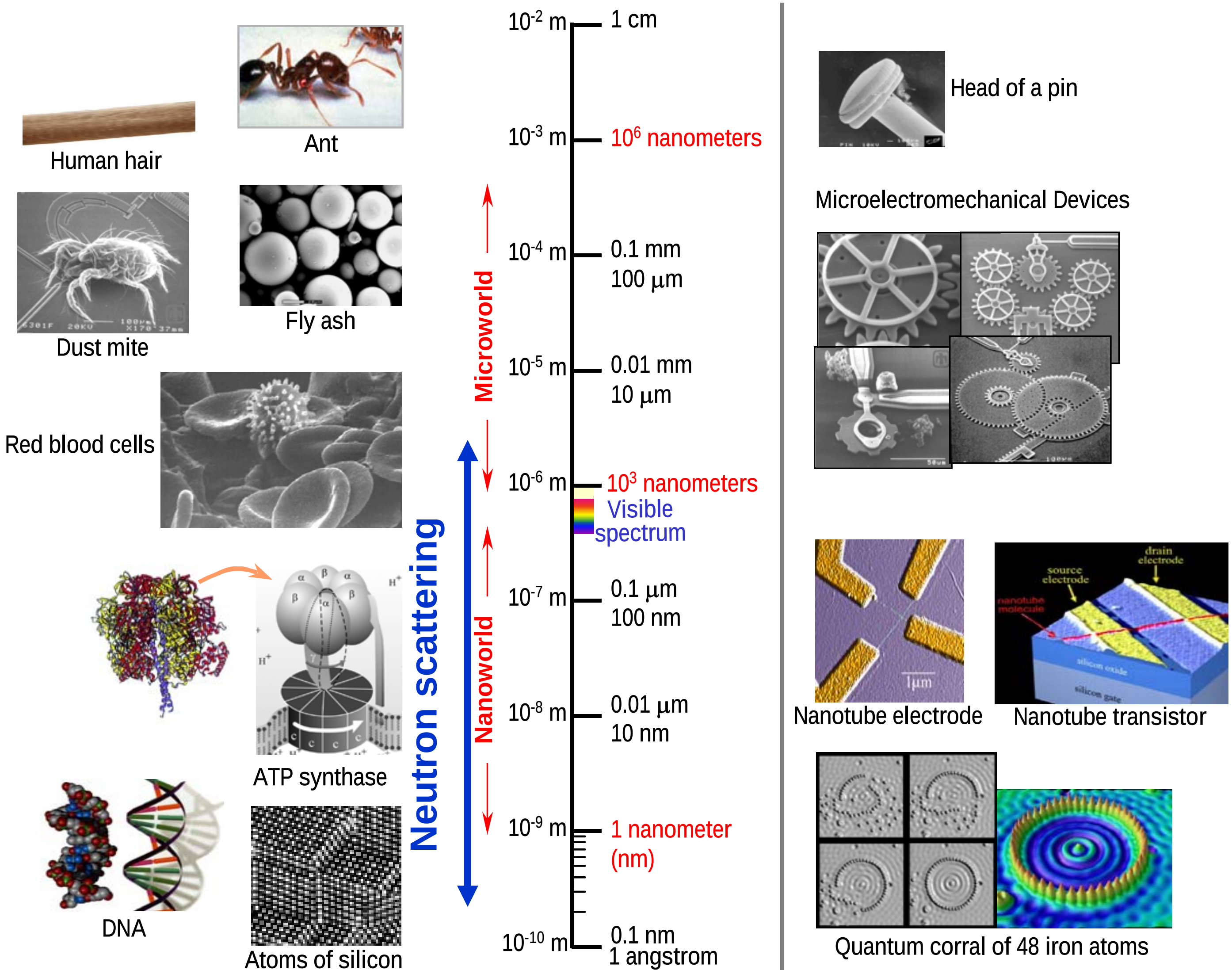
HFIR



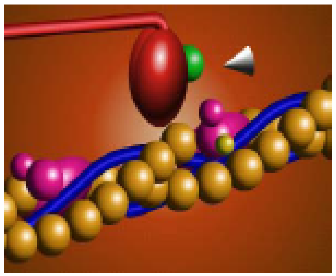
SNS

Neutrons: microns to angstroms!

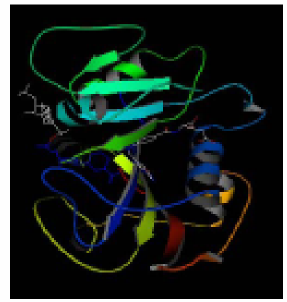
Bio – Materials



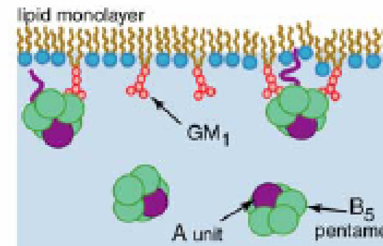
Present Areas of Biomolecular Research with Neutron Scattering



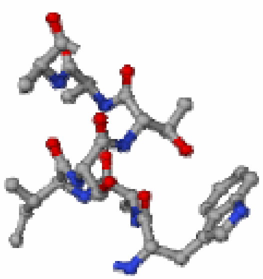
Biomolecular Complexes
Small Angle Neutron Scattering



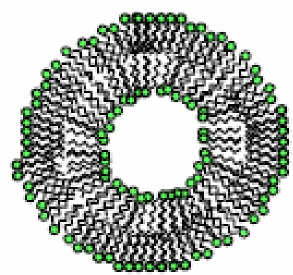
Protein Structures
Crystallography



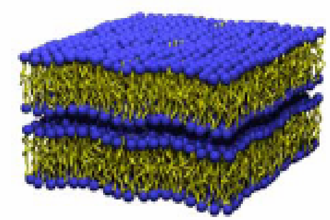
Layered structures
Reflectometry



Ligand Dynamics
Quasielastic scattering



Shape Fluctuations,
Polymer reptation etc



Collective Dynamics
Inelastic scattering

Neutrons in Biomedicine & Bio-Materials Opportunities & Applications

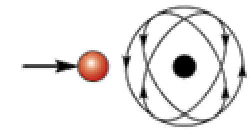
- Biomedical - protein structure and function – molecular machines and cell biology

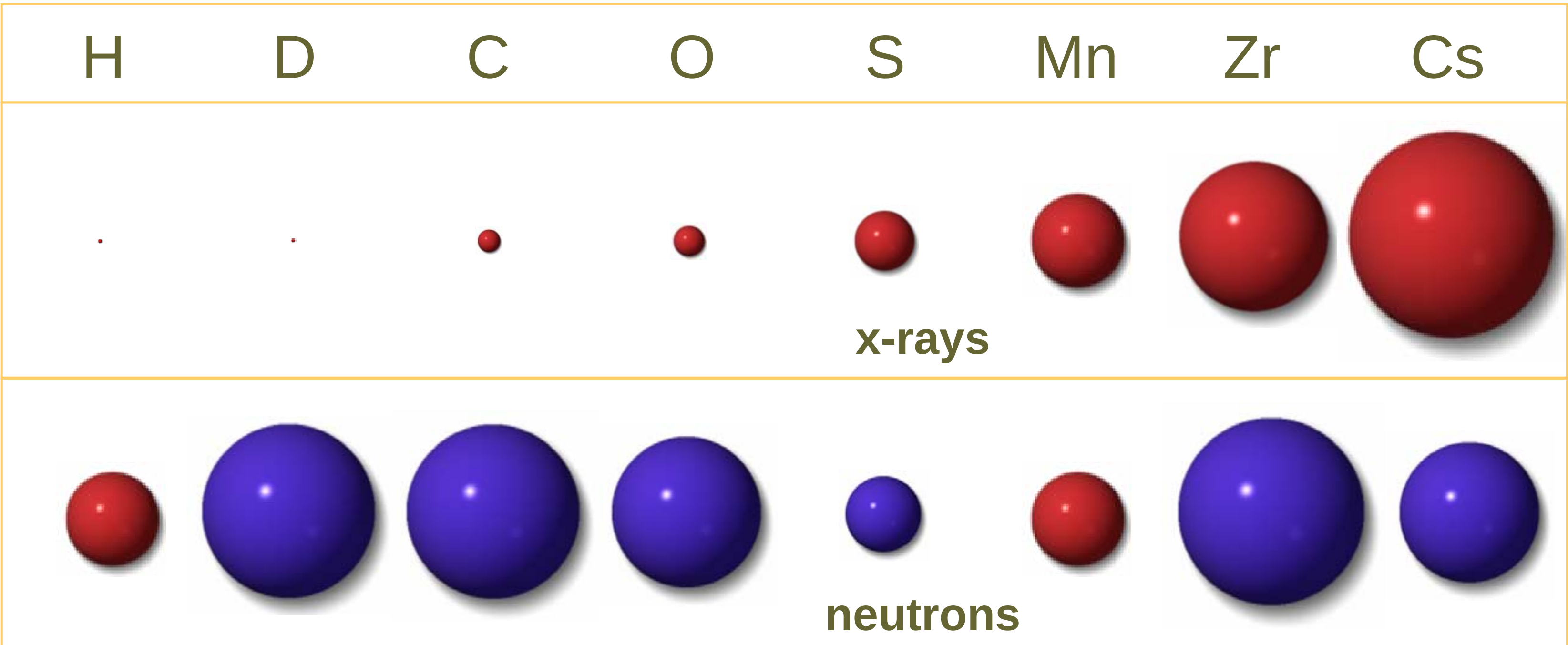
- Medical research - microbiology, disease mechanisms, high resolution imaging

- Advanced Bio-materials – nano-structured materials, bio-mimetic devices and bio-inspired materials

- Bio-Fuels & Energy – Bio-mass – informing process design and engineering, Bio-inspired & mimetic catalysts

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

Neutrons and Biology:

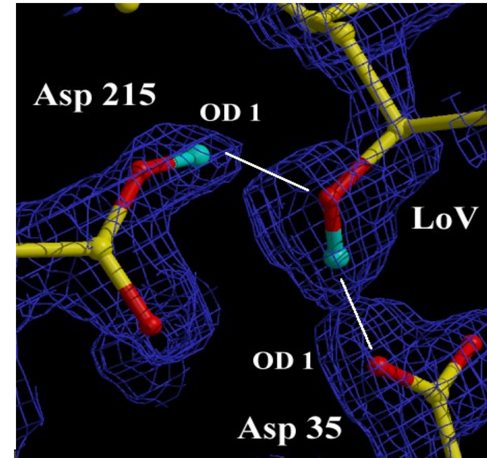
ORNL will provide world-leading instruments for neutron scattering at HFIR and at SNS

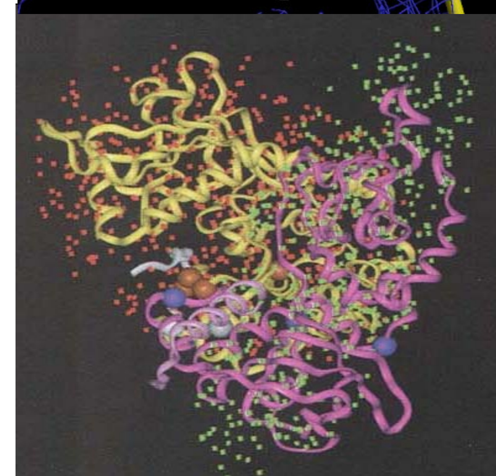
Neutrons are excellent probes for Hydrogen – and can discriminate between hydrogen and deuterium

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

Structure: H/D Labeled protein in complex systems

Dynamics: Specific H-Labeling in deuterated systems





Hierarchical Structures MEMBRANE ORGANIZATION

Fluid Mosaic Model – is the basic paradigm for the organization and dynamics of biological membranes.

Core structure of biological membranes is the phospholipid bilayer or the "membrane bilayer".

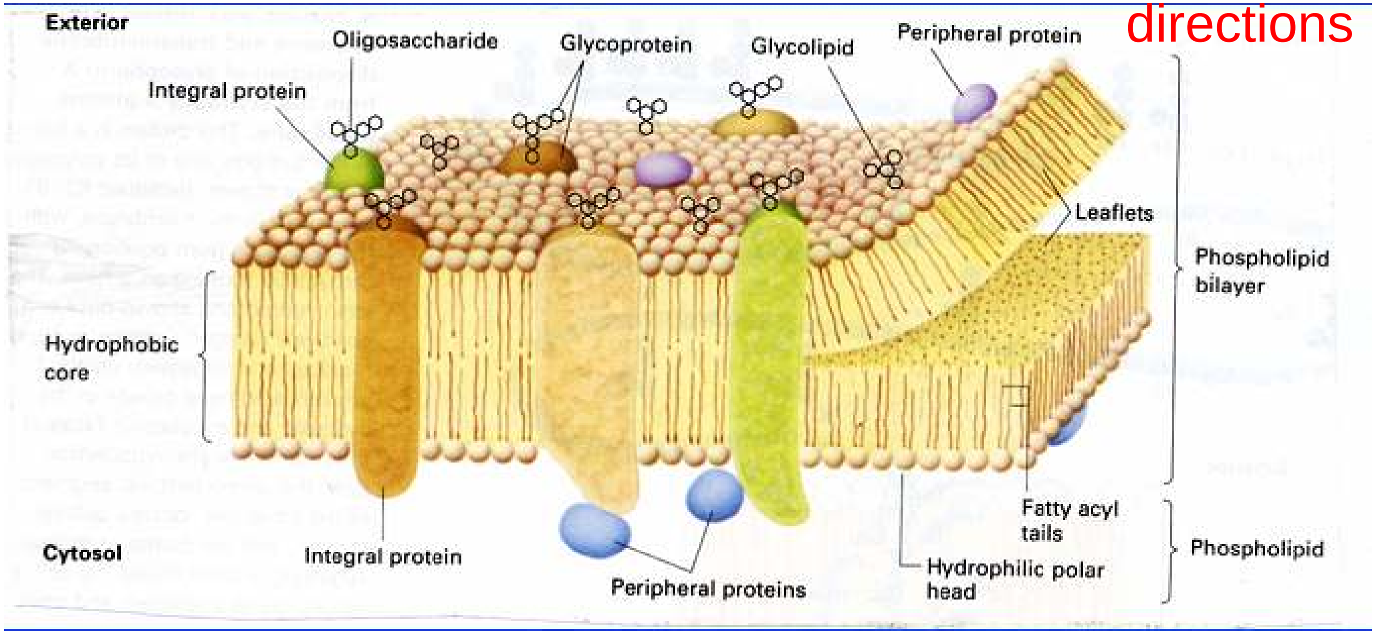
specular: profile in z-direction
off-specular: profile in x, y and z-directions

NR

SANS

NX

QENS



low resolution structure

high resolution structure

molecular dynamics

Structure of a biological membrane

The ORNL Center for Structural Molecular Biology
An Integrated Platform for Structural Biology

Bio-SANS at HFIR
- Macromolecular complexes

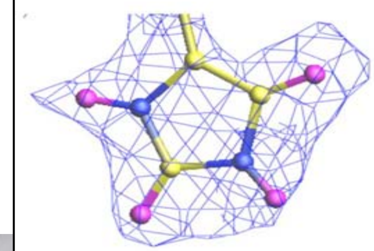
Membrane Diffraction
- Bio-mimetic membranes & systems

Neutron Protein Crystallography
- Protein structures at atomic resolution

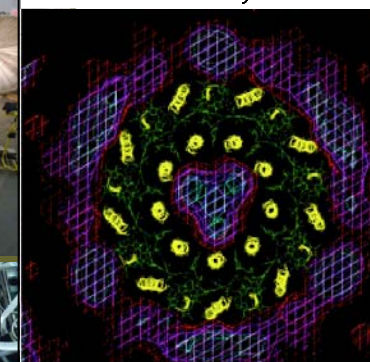
Bio-Deuteration Laboratory
- H/D-labeled proteins & molecules

Computational Methods
- Modeling protein complexes

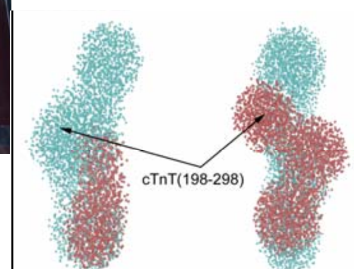
Supporting Instrumentation
- X-ray crystallography, SAXS, light scattering, spectroscopy



Bio- catalysis



Membrane proteins

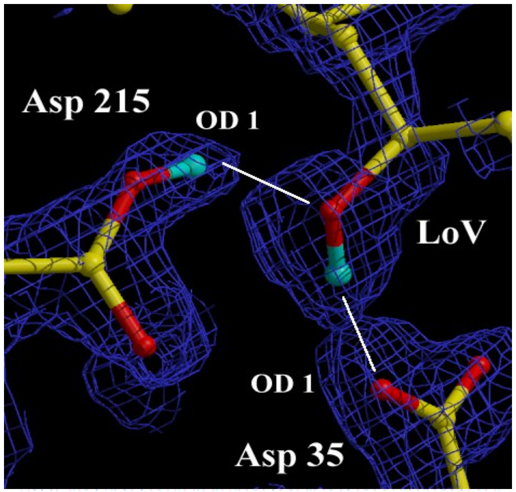


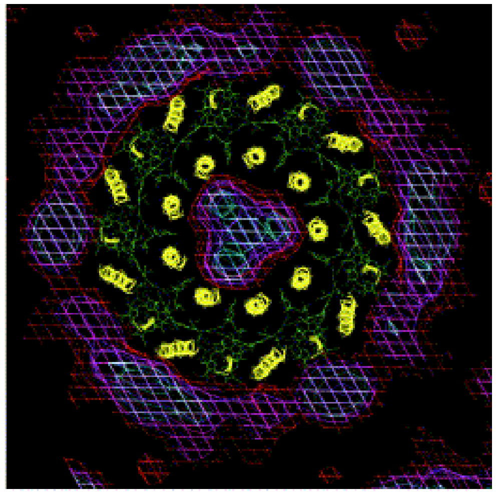
Protein Complexes

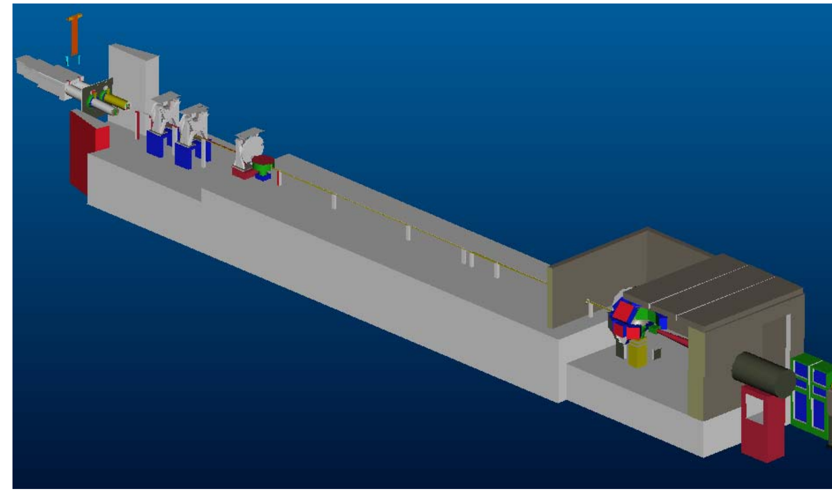
MaNDi – Macromolecular Crystallography at SNS

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

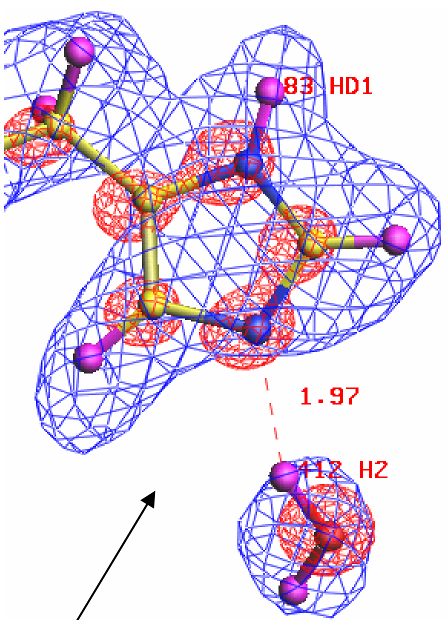
- Optimized for larger proteins and complexes
- High data rates (10 to 50X of existing facilities) and high resolution
- 1 mm^3 crystals with lattice repeat up to 150 Å and *d*min = 2.0 Å
- 0.125 mm3 crystals of deuterated proteins

Protonation state of histidine residues

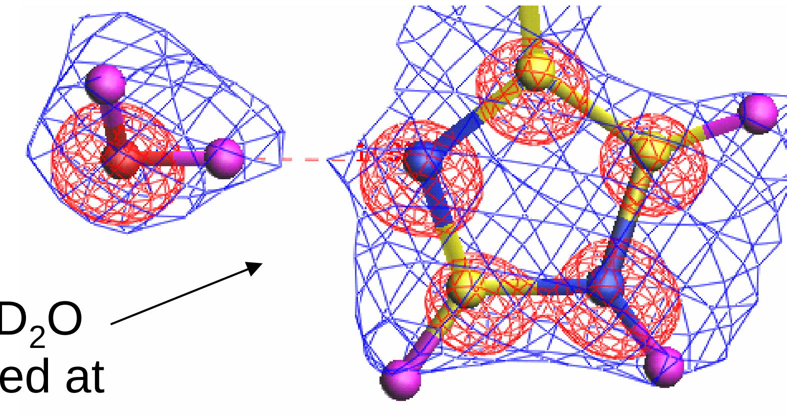
Blakeley et al, 2006

$2F_o - F_c$ positive neutron maps in blue

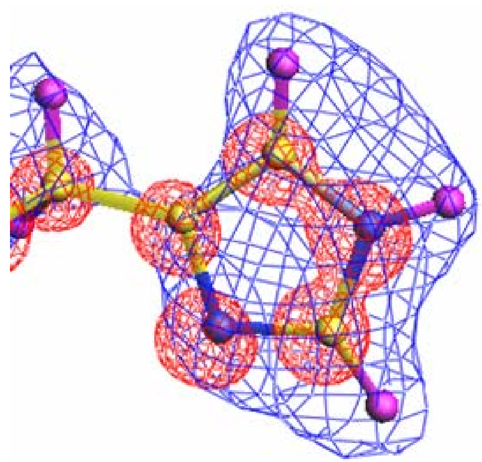
$2F_o - F_c$ X-ray electron density maps in red.



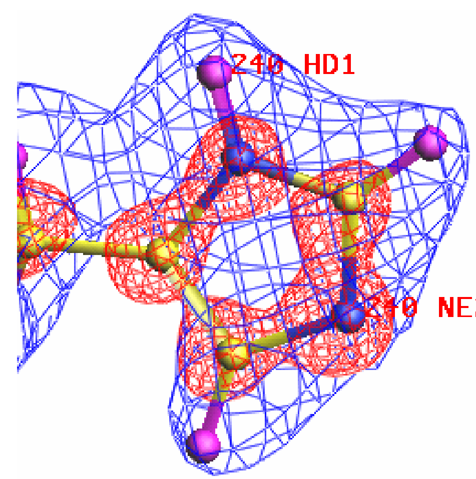
Histidine 83 + D_2O
(protonated at ND1)



Histidine 110 + D_2O
(Singly protonated at NE2)



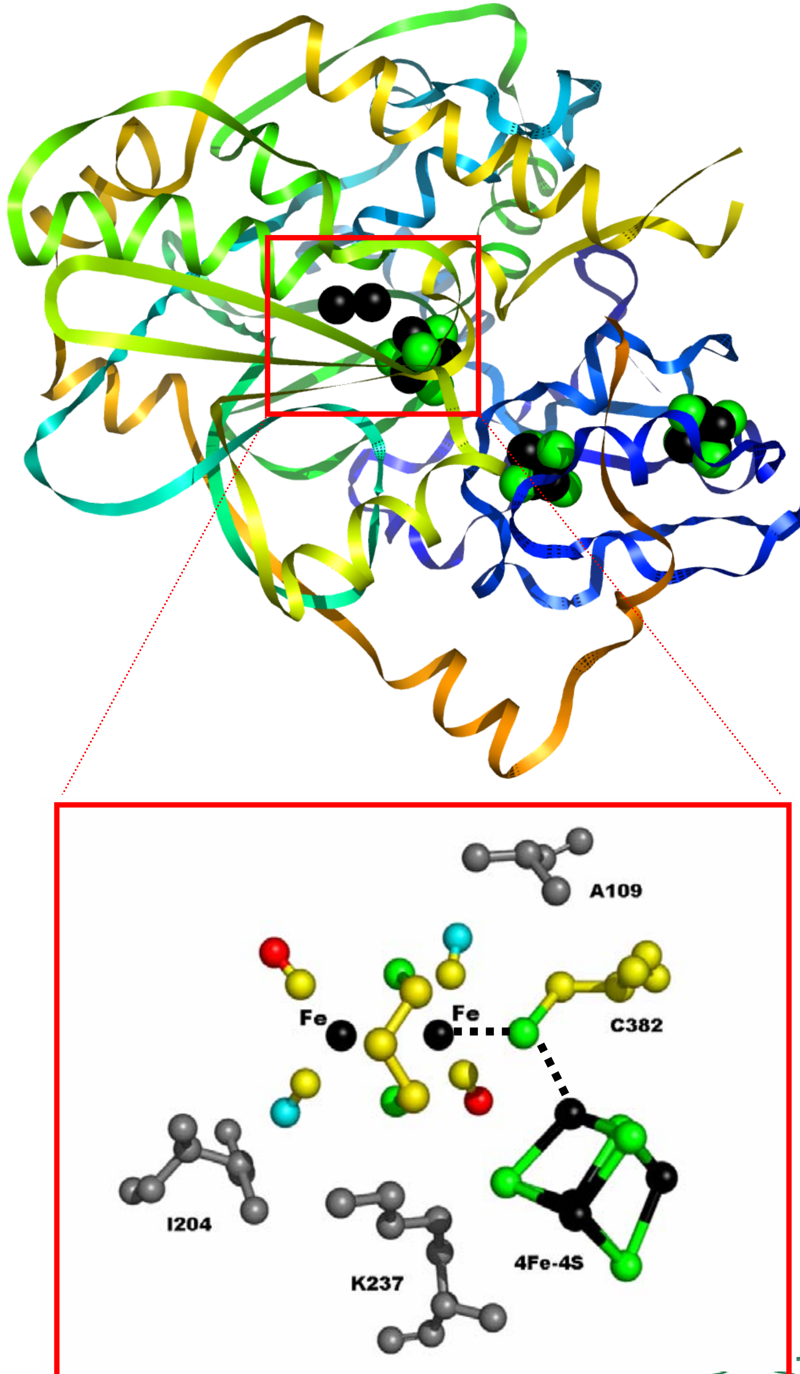
Histidine 187
(protonated at NE2)



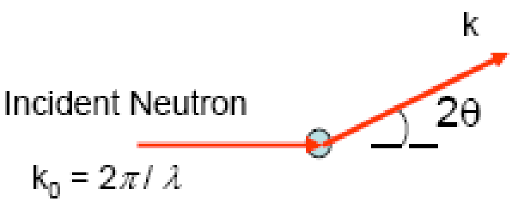
Histidine 240
(protonated at ND1)

Guiding Design: Bio-inspired Catalysis

- Hydrogenases:



Meso -> Macro Structures
Assemblies, Complexes, Composites



Momentum Transfer :

$Q = |\mathbf{k}-\mathbf{k}_0| = 4\pi \sin\theta / \lambda$

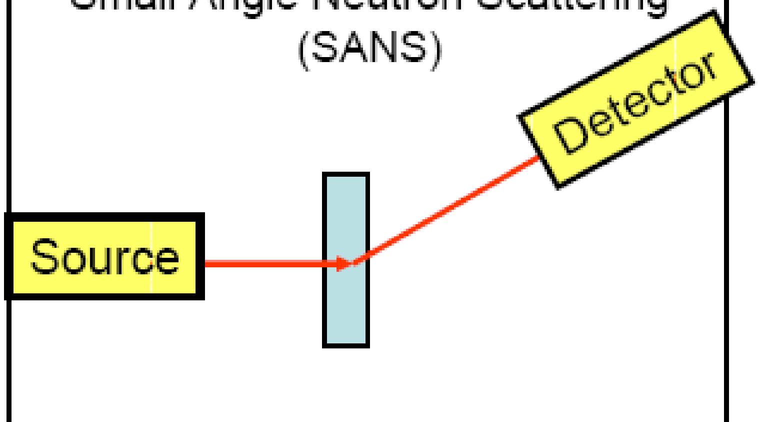
Scattering Amplitude:

$$A(\mathbf{Q}) = \int \rho(\mathbf{r}) e^{-i\mathbf{Q}\cdot\mathbf{r}} d^3 r$$

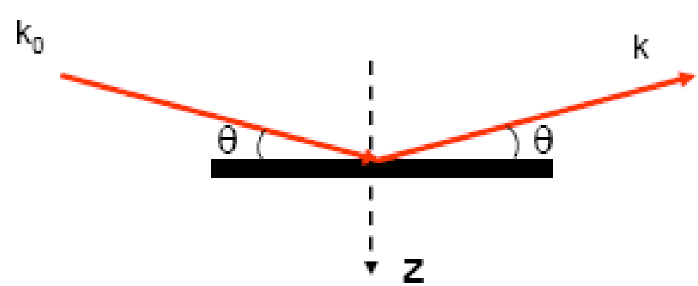
Scattering Intensity:

$$I(\mathbf{Q}) = |A(\mathbf{Q})|^2$$

Small Angle Neutron Scattering (SANS)



Reflectometry



Complex Structures

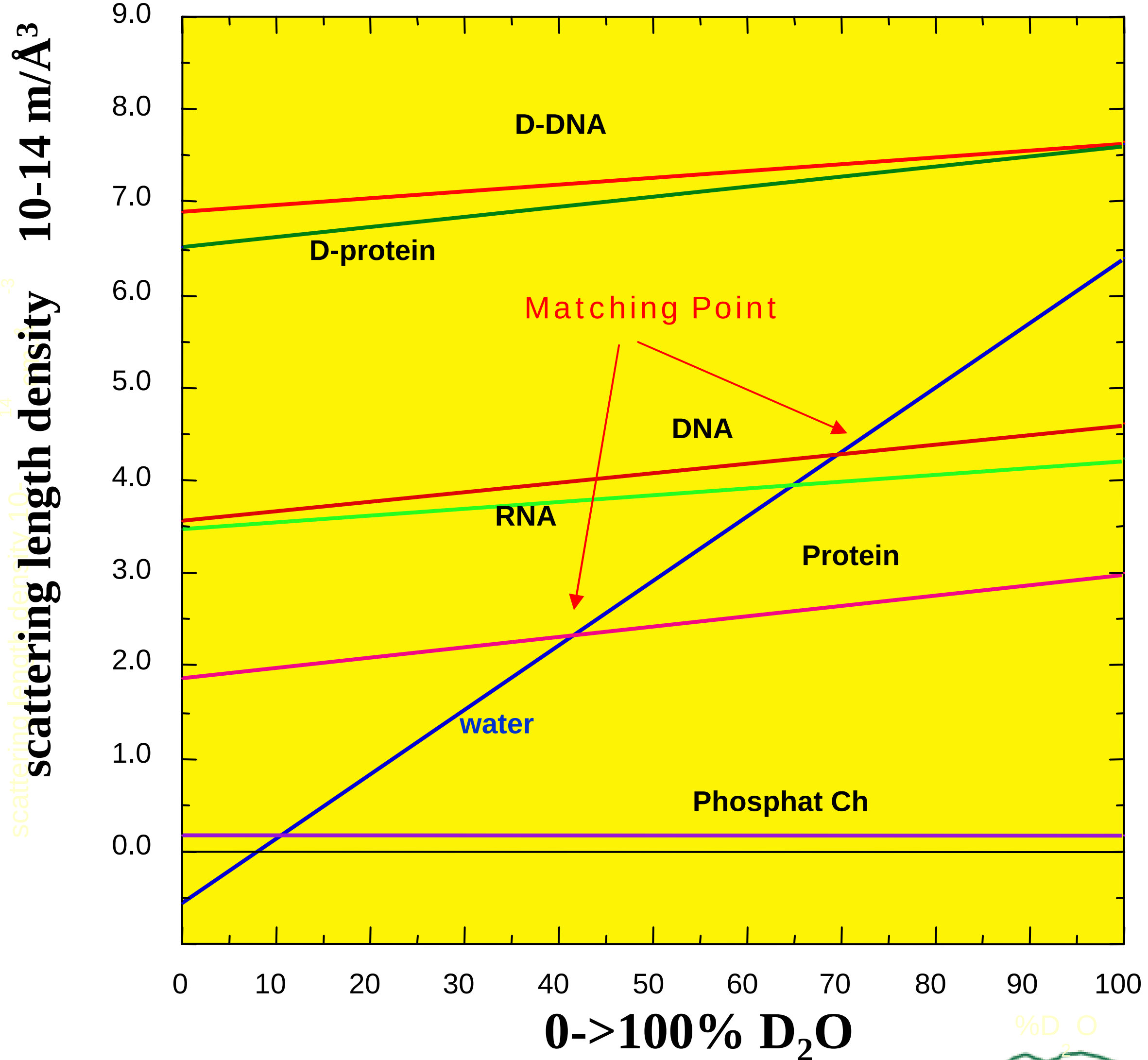
Low-Resolution Techniques provide molecular envelope shapes

Contrast variation

H_2O ~ - 0.5
D_2O ~ + 6.0

H_2O-D_2O fractions can be tuned and matched to 'contrast out' scattering from components in complex systems, assemblies and composites.

Contrast Variation



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

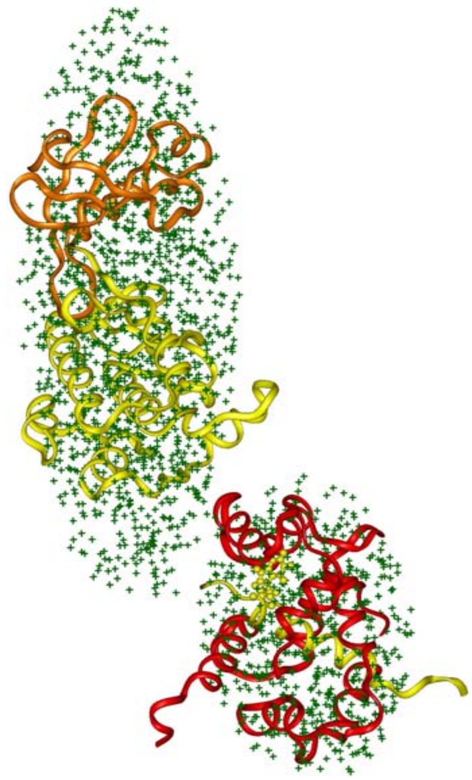
solution-state

useful range 10-1000Å (~15 – 500 kDa)

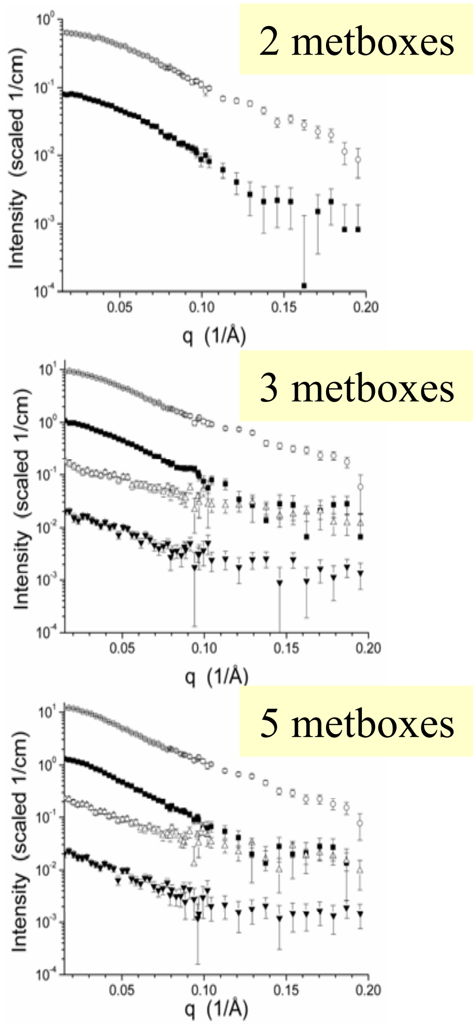
Visualizing interactions:

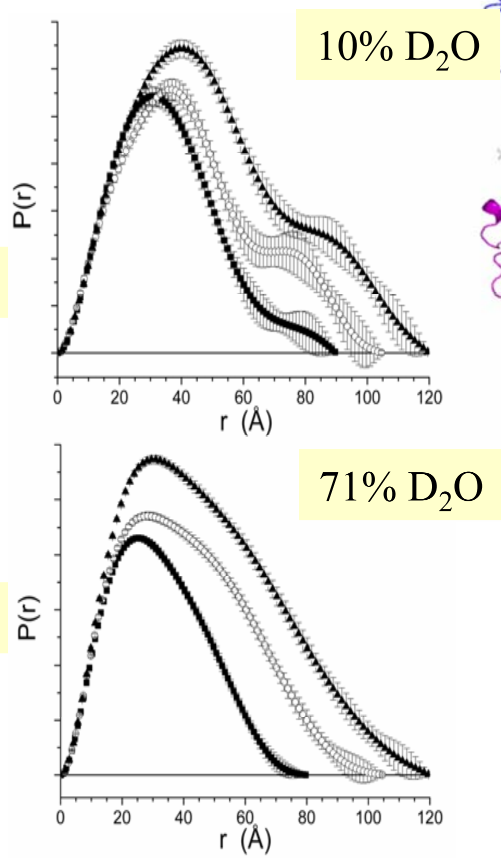
- 15-30A maps of complexes to identify relative positions of the individual components (interaction surfaces)

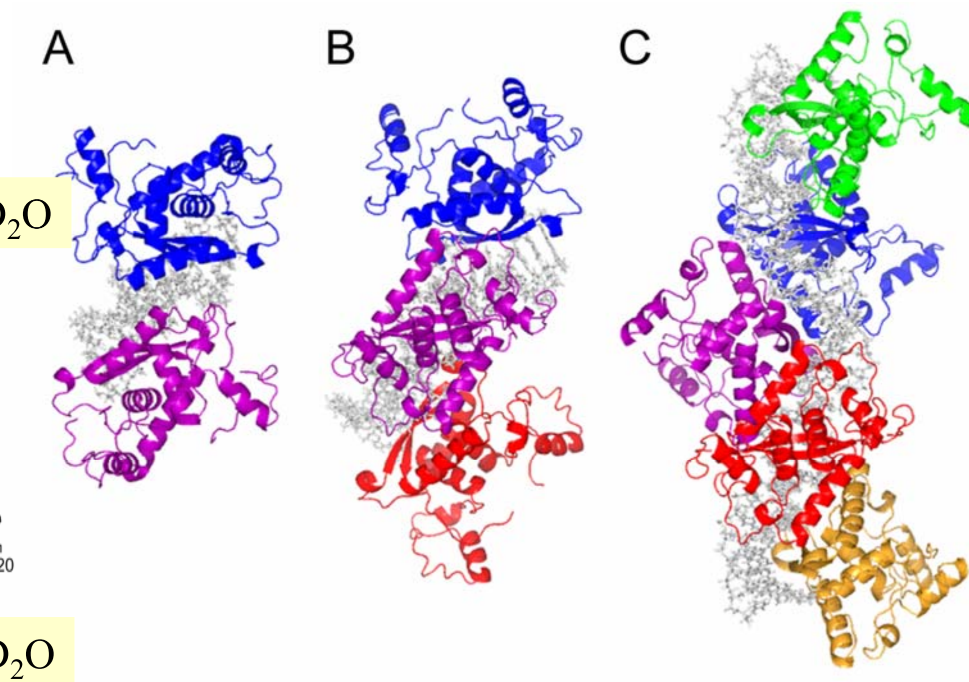
- reconstruction of the high-resolution image from the X-ray structures of the individual components



SANS of Methionine Repressor DNA complexes





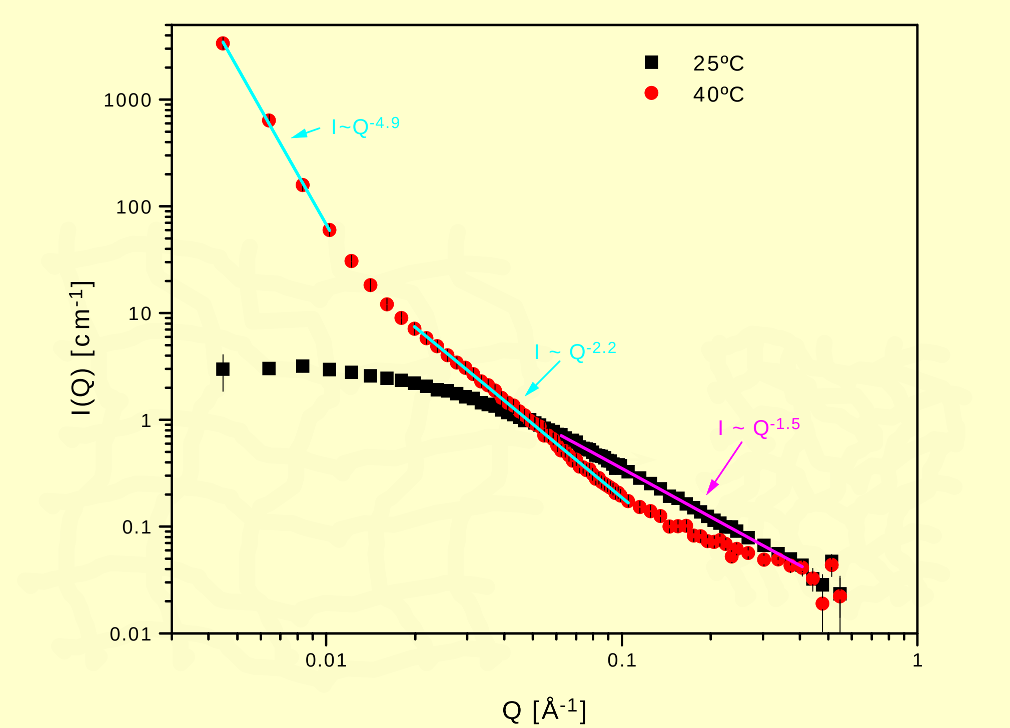


The protein packs very differently on the longest strand of DNA

Augustus, A. M., Reardon, P. N., Heller, W. T. and Spicer, L. D., J. Biol. Chem, 2006.

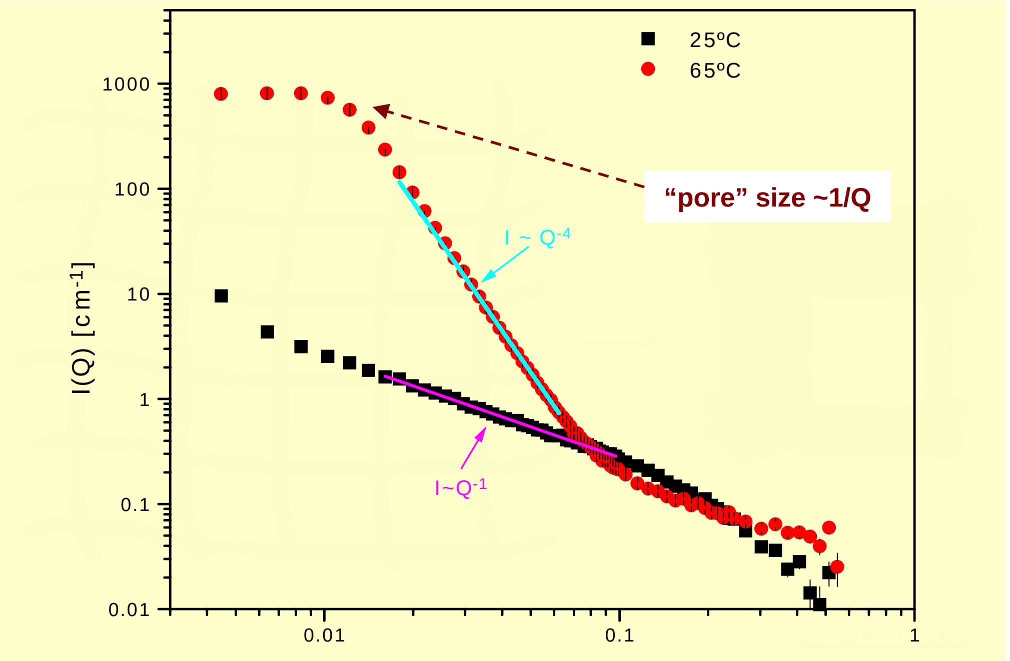
How SANS shows polymer morphology

PNIPAAm
poly(N-isopropylacrylamide)
25ºC - swollen
strands = *self avoiding path*
40ºC – *“homogenous” collapse*
- **strands ~ *random coil***
- **loss of permeability**



HPC
Hydroxypropyl cellulose
25ºC - swollen
strands = stretched rods

65ºC - *“heterogeneous“ collapse*
- **strands = 3-dim aggregates with smooth surface**
- **permeability retained**



Synthesis and Neutron Scattering Characterization of Ordered Self-Assembled Polymer Nanostructures and Bio-membranes

Opportunities for block copolymers with tunable...

Molecular architecture

Phase morpholgy

Functional properties

Examples

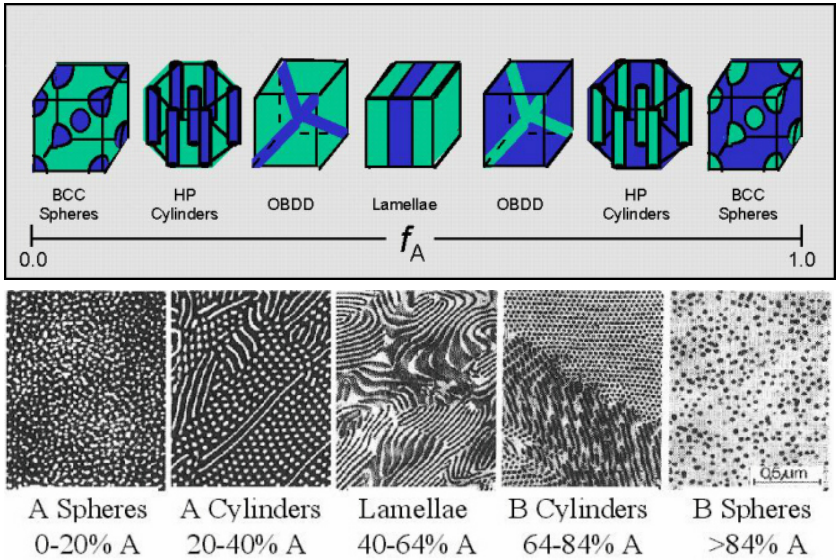
LEDs: trap exciton at heterojunction

Photovoltaics: need distributed heterojunction

Needed

Mechanisms to manipulate phase orientation at will

Basic understanding of macromolecules in oriented nanophases



Volker S. Urban, Kunlun Hong, Phillip F. Britt,

Chemical Sciences Division

Jimmy W. Mays, Distinguished Scientist

UTK & Chemical Sciences Division

Alexander Böker, *Lehrstuhl für Physikalische Chemie II, Universität Bayreuth, Germany*

Design, Structure and Function in Polymeric Biomaterials

- Natural – cellulose, sodium alginate, and natural rubber
- Animal materials – tissue based heart valves and sutures, collagen, glycosaaminoglycans (GAGs), heparin, and hyaluronic acid
- Nucleic Acids – DNA/RNA
- A wide variety of synthetic polymers
  - PMMA- hydrophobic (bone cement ingredient for orthopedic implants)
  - PHEMA – soft contact lenses
  - PAGE- Biomedical separations
  - PAA- Dental cements; mixed with inorganic salts; also used in mucosal drug delivery applications
  - PE (HD) –artificial hips & other prosthetic joints
  - PP- sutures & hernia repair.

Bio-SANS at HFIR – Dedicated to Biology

Bio-Macromolecules & Assemblies

Multi-Protein complexes
Protein/DNA complexes
(Lipids, Carbohydrates)

Hierarchical biological structures
Gels, Fibers & fibrils
Vesicles, Microemulsions
Membrane diffraction
Biomimetic & bio-inspired systems

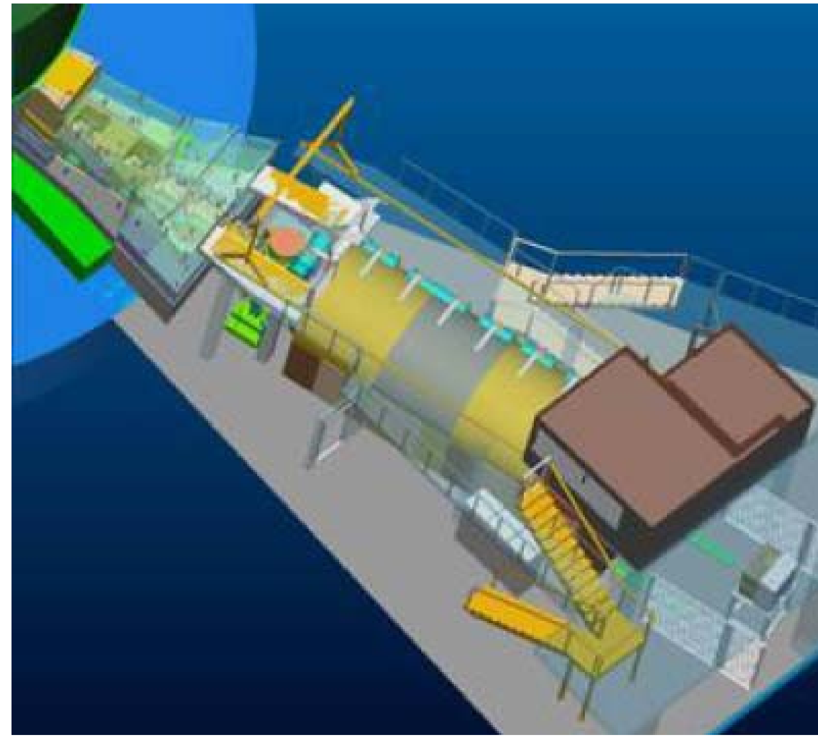
User Program, Infrastructure & Support

- Bio-SANS Lab for bio-sample preparation
- DLAB - H/D-labeling, isolation & characterization
- Computational tools for structural biology
- New SAXS and Light Scattering

Specifications	
Wavelength	6< λ <30 Å
Wavelength resolution	$\Delta\lambda/\lambda$ = 8–45%
Flux on sample	10^7 - 10^8 n/cm²/s
Q-range	0.002 - 1 Å^{-1}
Sample-detector distance	1 - 15 m
Detector	2-D ^{3}He detector
Detector size	1m x 1m
Detector resolution / pixel size	5.1x5.1 mm²
Max count rate	200 kHz

EQ-SANS: Extended Q-Range SANS

J.K. Zhao, (865)574 0411, zhaoj@ornl.gov



Main Features:

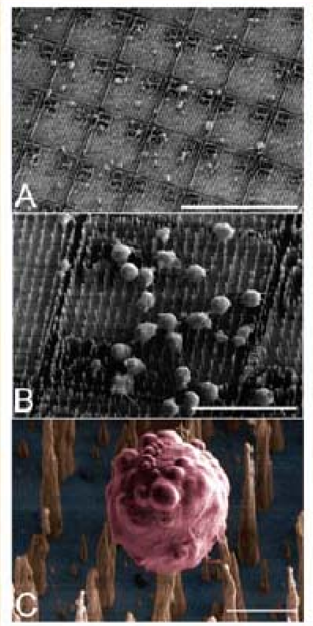
- Multiple length scale –
  - covers four decades in Q-range
  - (0.001-10Å^{-1})
- High intensity
- High wavelength-resolution

Example Applications

- Material Science: Simultaneous monitoring of domain structure at low-Q and crystalline lattice structure at high-Q.
- Life Science: Protein-membrane interaction, with protein structure shows up at low-Q and membrane structure at high-Q.

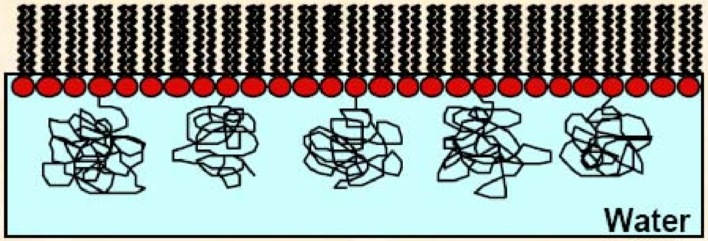
Biomimetic Membranes and Materials

Rapidly expanding field at the interface of materials science, Nanoscience and biology

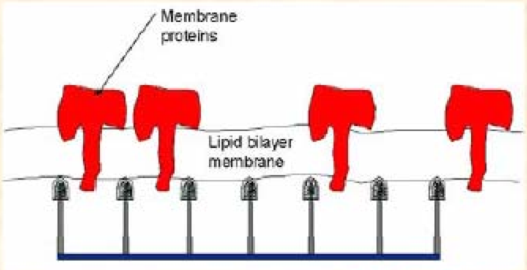


As new materials and technologies are developed, characterization tools are needed to study the structure and function of:

- Supported Membranes
- Polymer/Biological Macromolecule composites
- Medical Materials
- Biosensors



Macromolecular Complex Systems and Nanofabrication themes of the Center for Nanophase Material Science have interests in these areas.

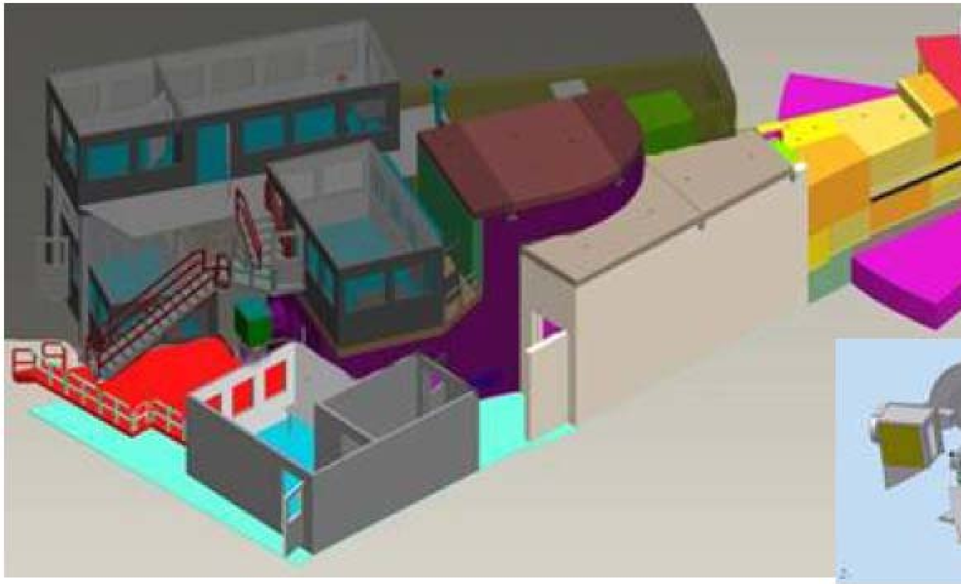


Liquids Reflectometer

John Ankner, *(865)576 5122, anknerjf@ornl.gov*

Main Features:

- Optimized for air, liquid, and solid interfaces studies
- Off-Specular reflectivity
- and in-plane scattering studies
- 1-2 orders of magnitude faster



Example Applications

- Membranes and their intermolecular interaction
- Protein adsorption on surface
- Phase separation in polymer films
- Surfactants at interfaces
- Interfacial structure in drug delivery systems

Bio-medical Applications:

- Phase separation in polymer films
- Templating at air/water interfaces
- Complex fluids under flow
- Vesicles and gels
- Reaction kinetics
- Surfactants at interfaces
- Interfacial structure in drug delivery systems
- Membranes and their intermolecular interaction
- Protein adsorption
- Functionializied & Patterned Surfaces
- Biocompatibility and sensors

Deuteration and H/D-specific labeling

Neutron incoherent scattering background swamps the signal

coherent scattering : structural information

incoherent scattering: background

$$\left(\frac{\text{signal } I}{\text{noise } \sigma(I)} \right)$$

	C	N	O	H	D
bcoh (fm)	+6.65	+9.36	+5.81	-3.74	+6.67
σcoh	5.56	11.03	4.23	1.76	5.59
σinc (barns)	0	0.49	0	80.27	2.05

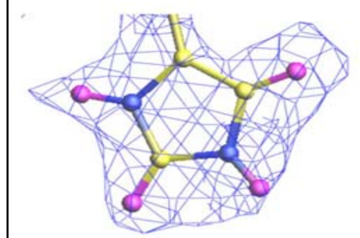
Exchange Deuterium for Hydrogen >> S/N

Bio-Deuteration Laboratory

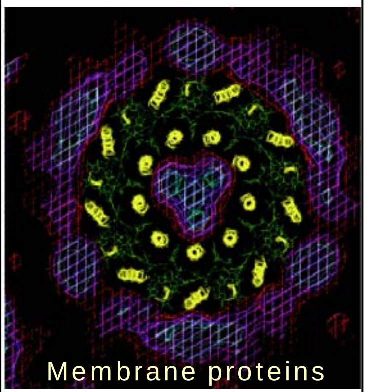
Central facility and user program for *in vivo* H-D labeling of macromolecules

- Develop a Central Deuteration Laboratory dedicated to specific H/D labeling of cells, proteins, nucleic acids and other bio-molecules.
- Develop better and faster systems and methods to produce deuterium labeled biological macromolecules for the biology community
- Improving downstream technologies to exploit these reagents (including data collection and interpretation for neutron scattering)
- Train research students and staff in application of these powerful techniques

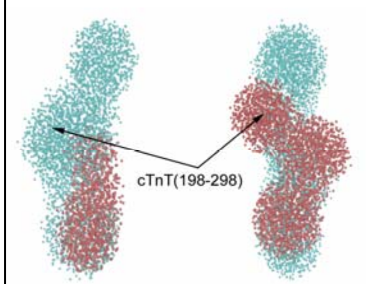
Similar User Facilities at EMBL-ILL and at LANL



Enzymes - catalysis

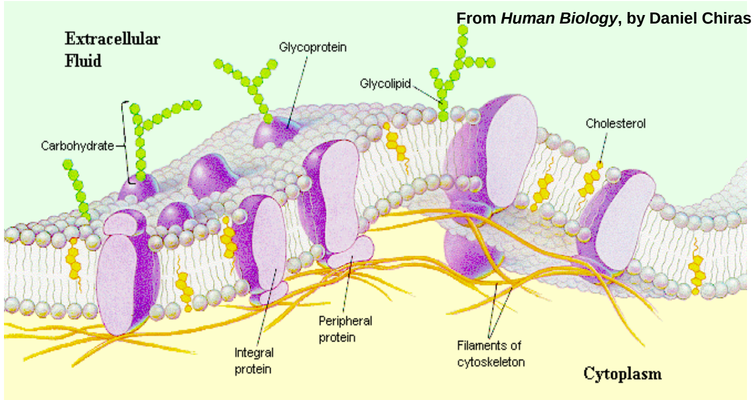


Membrane proteins



Protein Complexes

Membrane Proteins: Structure and dynamics



Biological membranes are complex mixtures of lipids, carbohydrates, and proteins embedded in fluid lipid bilayers

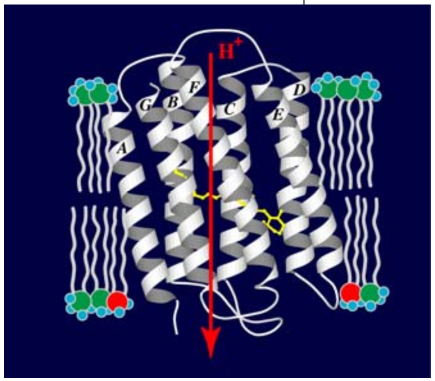
STRUCTURE:

H/D-labeling "highlights' components *in situ*

DYNAMICS: link structure & function

catalysis, regulation, transport, formation of assemblies, cellular locomotion

Reverse H-labeled groups in D-proteins allows neutron spectroscopy to study complicated biological phenomena in quantitative detail changes in 1000s of coordinates

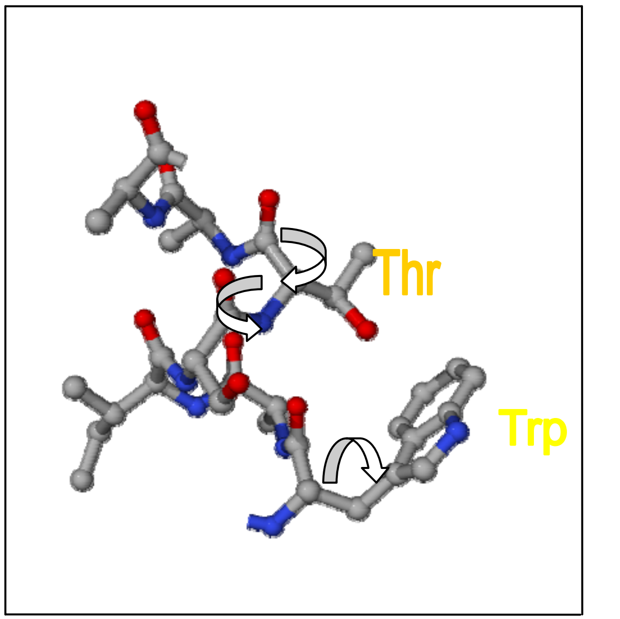


Neutron Spectroscopy: Protein Dynamics

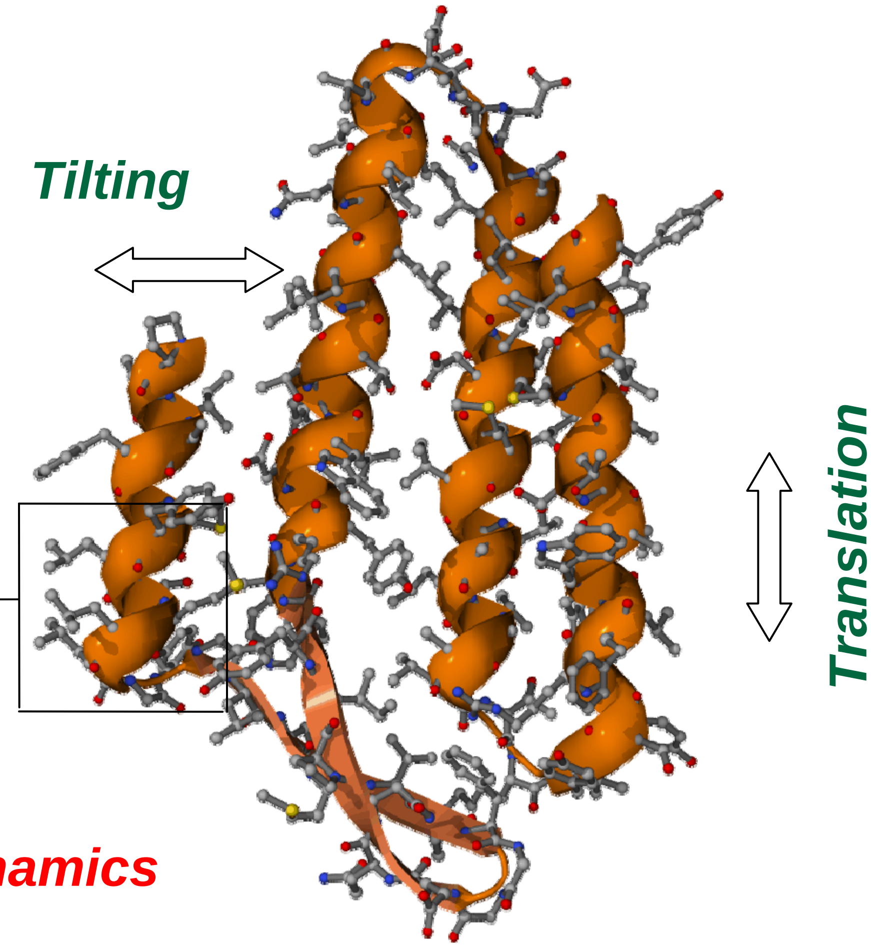
ORNL Participants

Dean Myles, Ken Herwig & Pratul Agarawal

Internal Dynamics



Global Molecule Dynamics

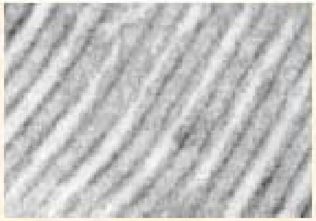


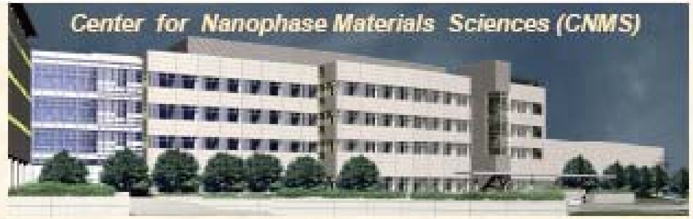
H-labeling - Residue Specific Dynamics
- one residue at a time

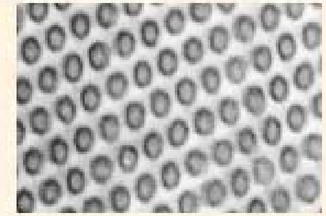
Rotation

Oak Ridge National Laboratory
U. S. Department of Energy

UT-BATTELLE



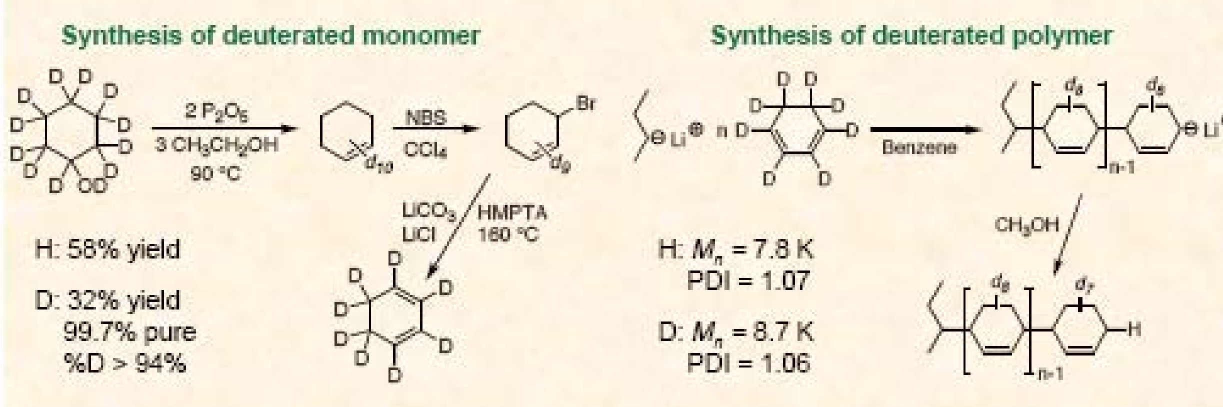




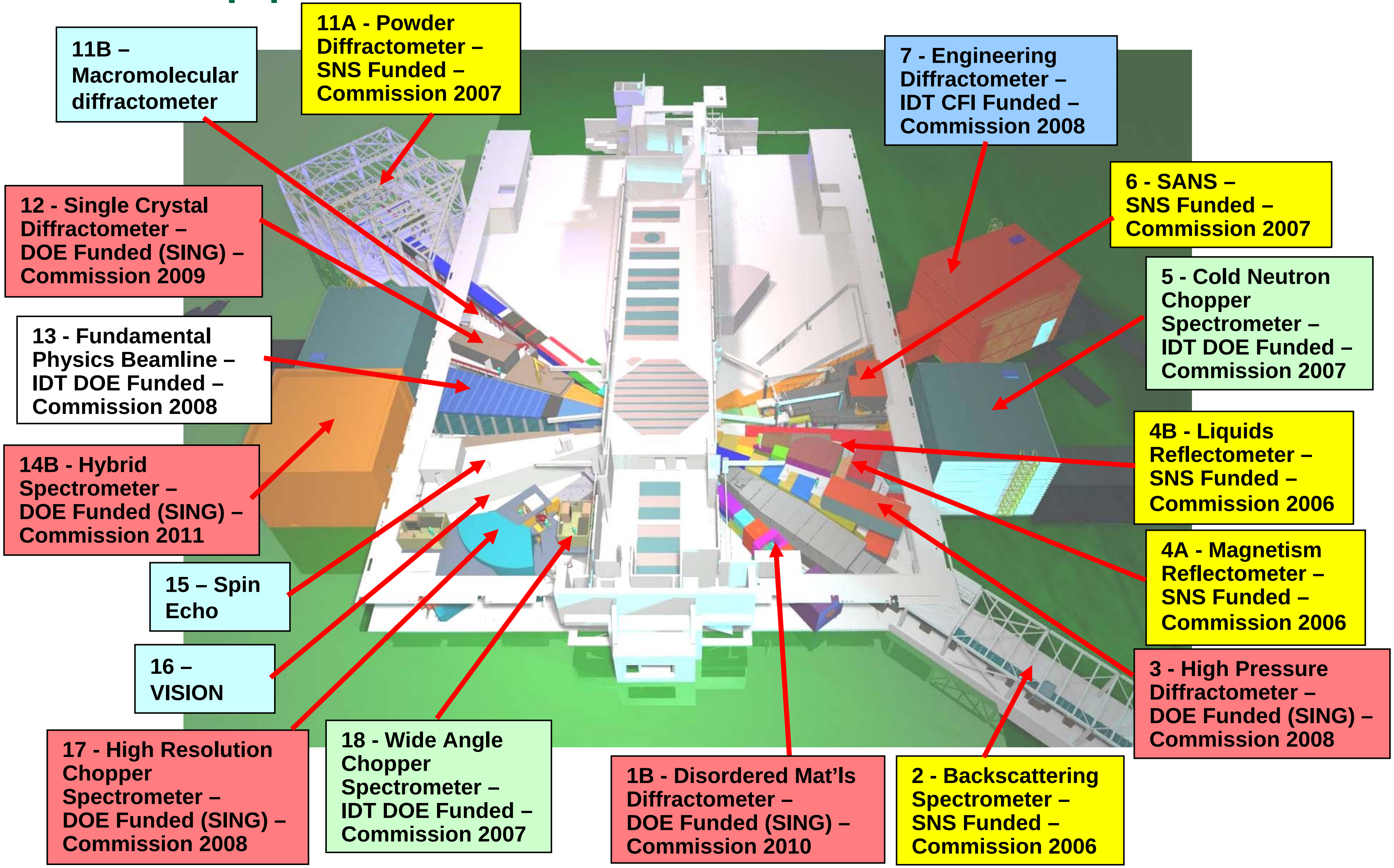
Capabilities and Research Opportunities

Unique Deuteration Capabilities Available

- **Synthesis and characterization of small molecules (monomer), polymers, and biomaterials**
- **Unique anionic synthetic techniques for the preparation of well defined polymers and complex polymer architectures (stars, combs, and hyperbranched polymers)**
- **Synthetic capabilities in preparation of novel polymer architectures based solely or in part on amino acids**
- **Expertise in directed self-assembly of molecules**
- **Unique capabilities in SWNT chemistry and in preparing and characterizing polymer-carbon nanotube composites**



17 Approved Instruments….



Summary

- Structure and dynamic information are key to addressing significant problems in bio-medical technologies
- The problem is complex – no single tool will provide all the necessary information.
- Neutron scattering and imaging provides information on length scales that are of interest to biology: From the atom to the cell to man !
- Neutron spectroscopy provides information on the pico-nano second length scales of interest to biochemical processes of life
- SNS and HFIR provide will provide new opportunities in scattering and imaging charaterization of bio-medical materials, complexes and assemblies

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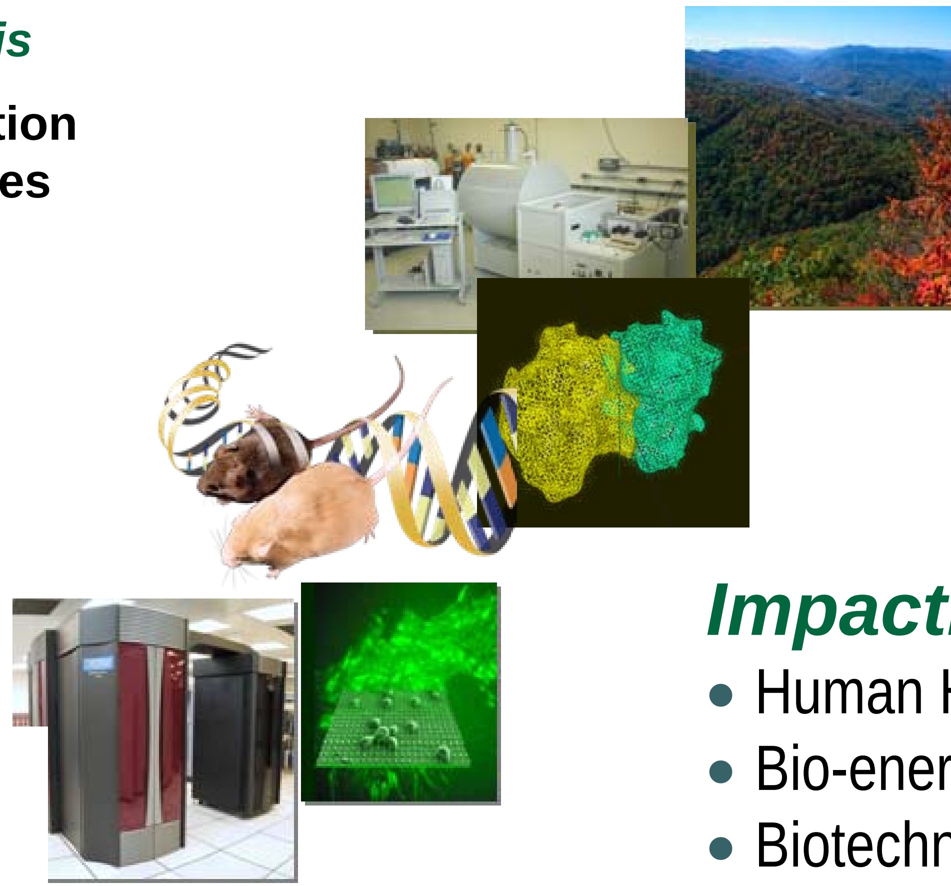
The ORNL Center for Structural Molecular Biology
An Integrated Platform for Neutron Structural Biology

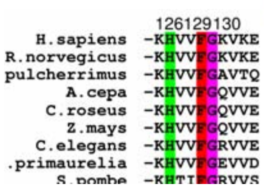
Bridging the gap between molecular and cellular structural analysis

Protein Structure & Function
Macromolecular complexes
Membrane complexes
Transient complexes

Partnering with:

Biological Science
Chemical Science
Computational Science
Neutron Science
Academia





Impacting

- Human Health
- Bio-energy
- Biotechnology
- Ecology