OAK RIDGE National Laboratory

Center for Structural Molecular Biology

Hugh O'Neill

Biological Labeling and Scattering Group Leader
Center for Structural Biology Director
Neutron Scattering Division
Oak Ridge National Laboratory

ORNL is managed by UT-Battelle, LLC for the US Department of Energy

BER Structural Biology Resources
U.S. Department of Energy

U.S. DEPARTMENT OF ENERGY

Center for Structural Molecular Biology

A DOE BER supported Structural Biology Resource

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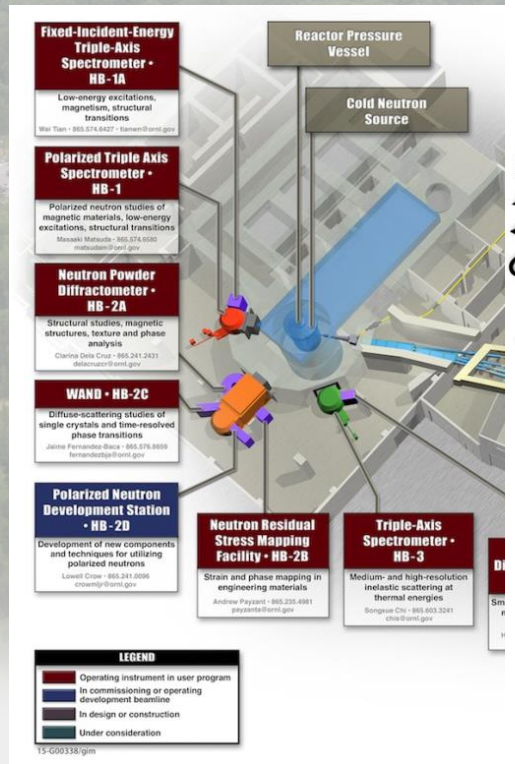
High Flux Isoto

Biological small-angle n
(Bio



Bio-SANS

Bio-deuteration

on Neutron Source

euteration Program

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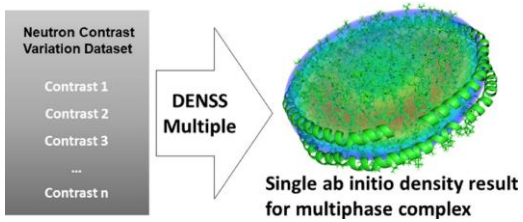
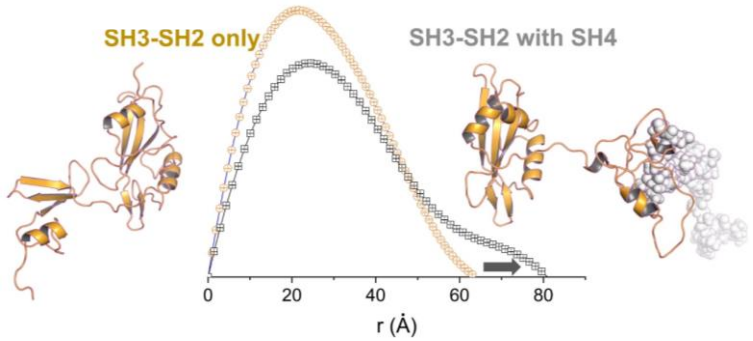
Understanding biological complexity;
A grand challenge for biological systems science

Biomacromolecules and complexes

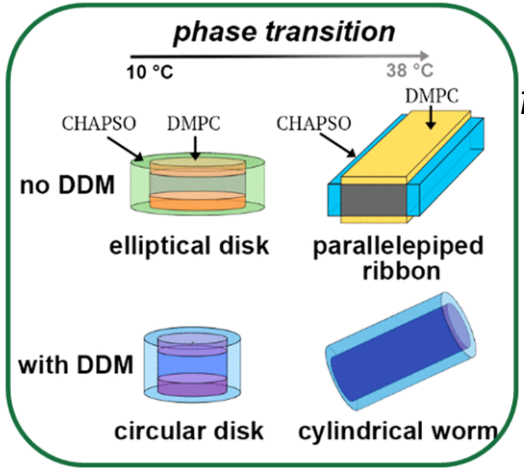


DENSS-multiple for modeling contrast variation SANS Sumner *BBA Advances*, (2022)



IDRs in multidomain proteins
Gurumoorthy *Biomac*. 2023

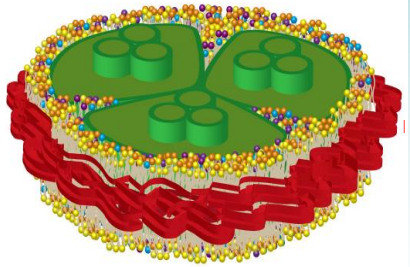
Biomembranes



Lipid bicelles for membrane protein stabilization

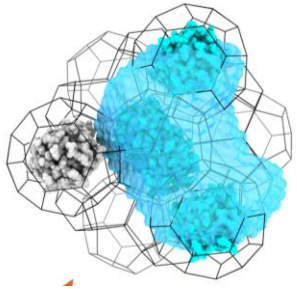
Leite *J. Phys. Chem Lett*. 2022

Membrane proteins under native conditions



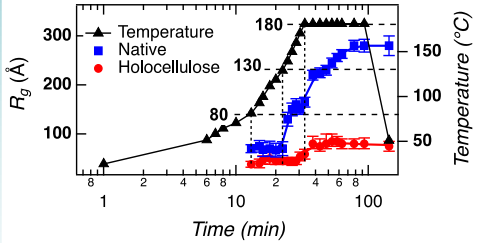
Brady *BBA Biomem.* 2022

Complex systems



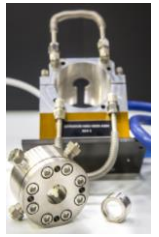
Proteins in Metal Organic Frameworks

Wang *Nat. Comm.* (2023).



Yang *ACS Sus. Chem. Eng.* (2022)

Pseudo-lignin in pretreated biomass



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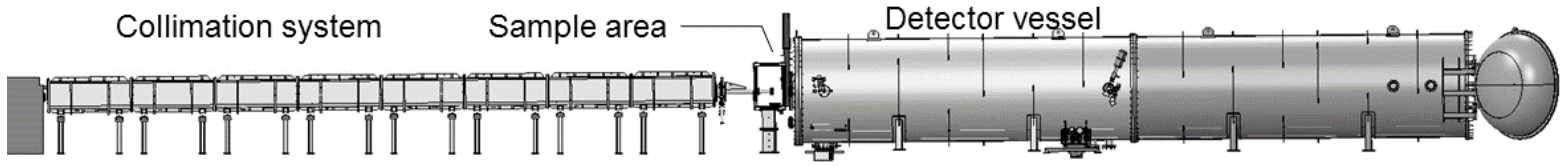
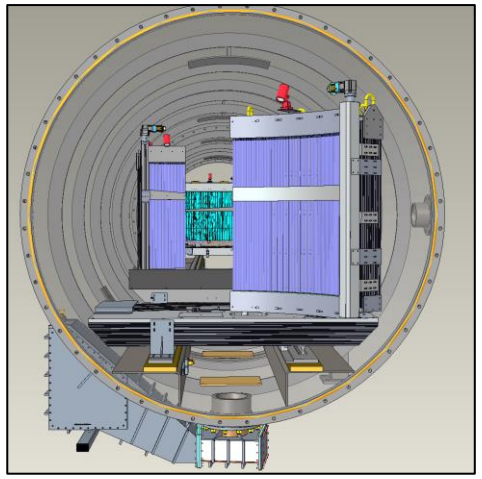
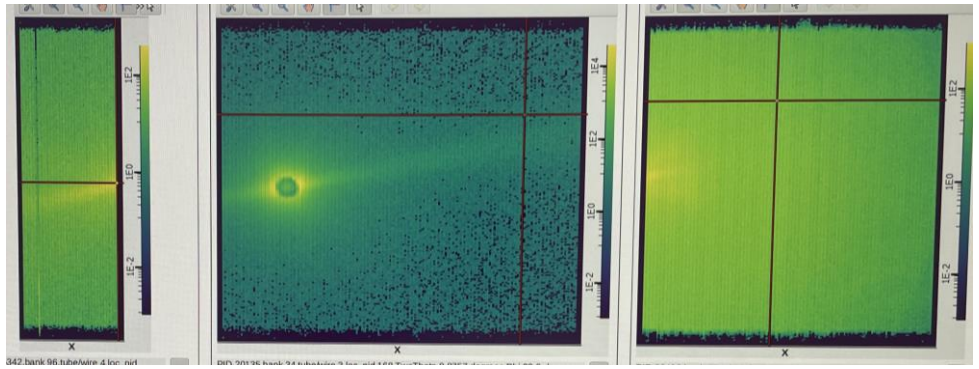
Bio-SANS



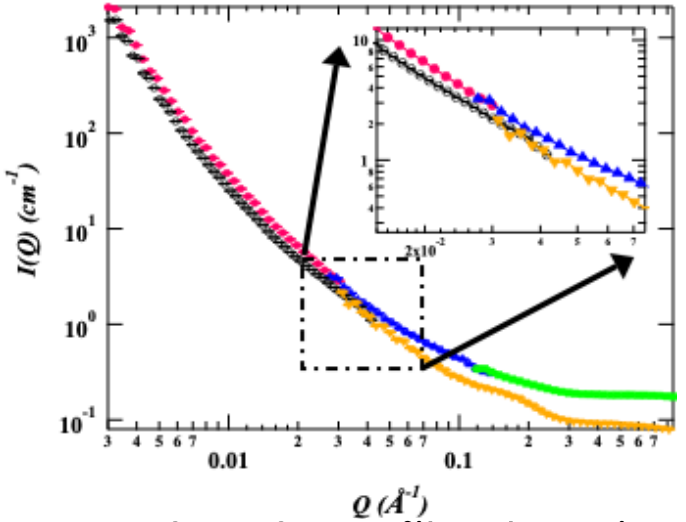
Three panel detector array



Detector layout in tank



Mid-range **Main** **Wing**



1D SANS profile showing overlap regions

- Improved data quality for hierarchical systems
  - Decrease Q-resolution mismatch and overlap data quality
- Increased angular coverage
- Enable sub-minute time resolution

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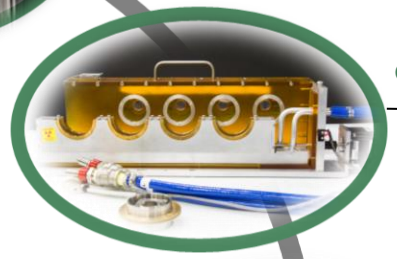
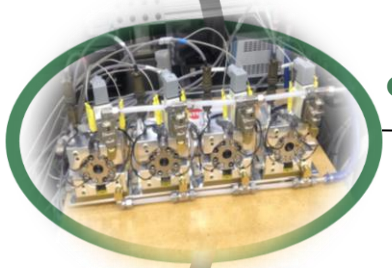
Sample environments for challenging biological problems

- **Robot sample changer**
  - ***Solutions in general, and gels, powders, and solid samples***

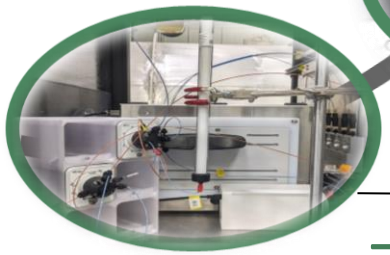


- **Tumbler**
  - ***Suspensions such as nanoparticles***



- **4-position pressure cell**
  - ***Biomass thermochemical pretreatment***

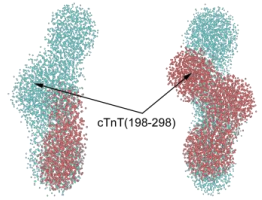
- **Grazing-incidence stage**
  - ***GISANS, membrane diffraction***

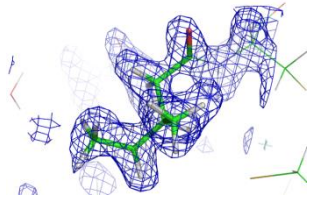


- **SEC-SANS**
  - ***Size-Exclusion Chromatography SANS***

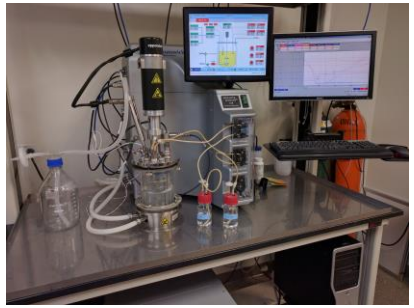
Bio-deuteration program

Neutrons are excellent non-destructive structural probes that can discriminate between hydrogen and deuterium.



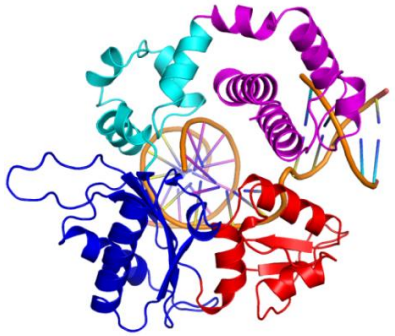


- Characterizing higher-order macromolecular complexes
- Pinpointing positions of individual hydrogen atoms
- Probing the structure and dynamics of biomacromolecules



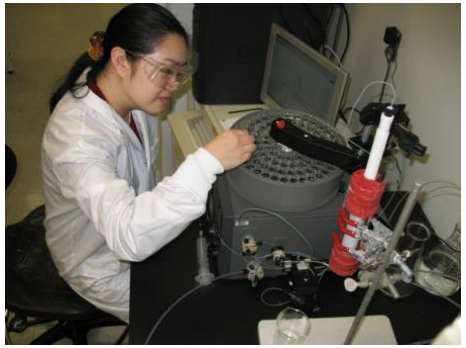
Fed-batch Fermentation

- *Escherichia coli*
- Yeast



H/D-labeled proteins

- Shake flask pro- and eukaryote expression
- Soluble/membrane proteins



Purification & characterization

- *Akta systems*
- *Spectroscopy*
- *GC-MS*

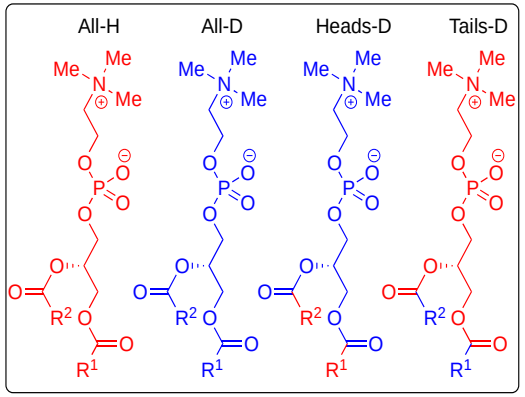
Biophysical techniques

- *SAXS*
- *Light scattering*

Lipid deuteration

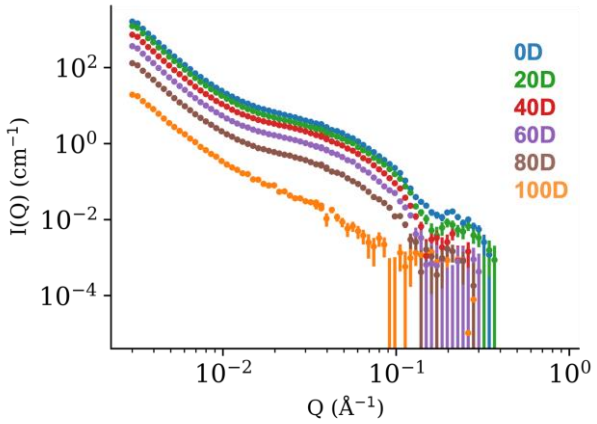
Unique insights into biomembrane properties and interactions with proteins and other molecules using neutrons

Deuteration strategy for D-incorporation in PC



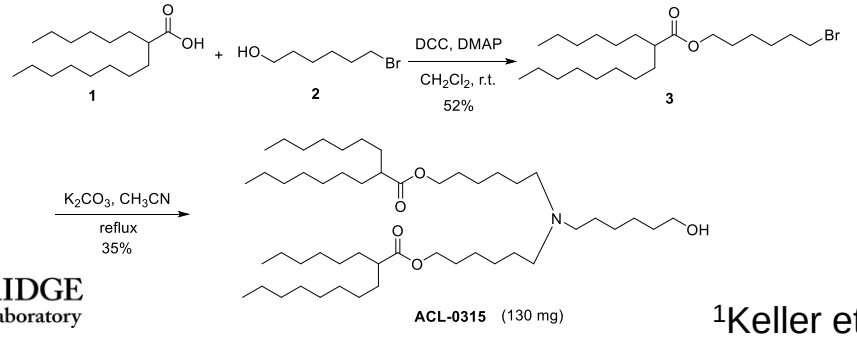
Moiety: Protiated Deuterated

SANS contrast series for deuterated PC



- Contrast match point 112.9% +/- 1.7 % D_2O

2-step synthesis of ionizable lipid ALC-0315



- Controlled D-incorporation into *E. coli* lipids[1]
  - MS and SANS characterization of D label distribution in PE and PG lipids
- Deuterated phosphatidylcholine from engineered *E. coli* expressing PC synthase
  - Synthesized deuterated choline
  - MS/NMR characterization
- Synthesis of deuterated ionizable lipids for lipid nanoparticles[2]
  - New synthetic route developed
  - Deuteration by D/H exchange in progress

[1]Keller et al. submitted

[2]In collaboration with Prud' Homme, Princeton U.

CSMB user program

General User Program

- 75% of available beam time
- 2 – 3 day experiments
- 2 proposal calls/year
- Request biodeuteration

Proof-of-Principle

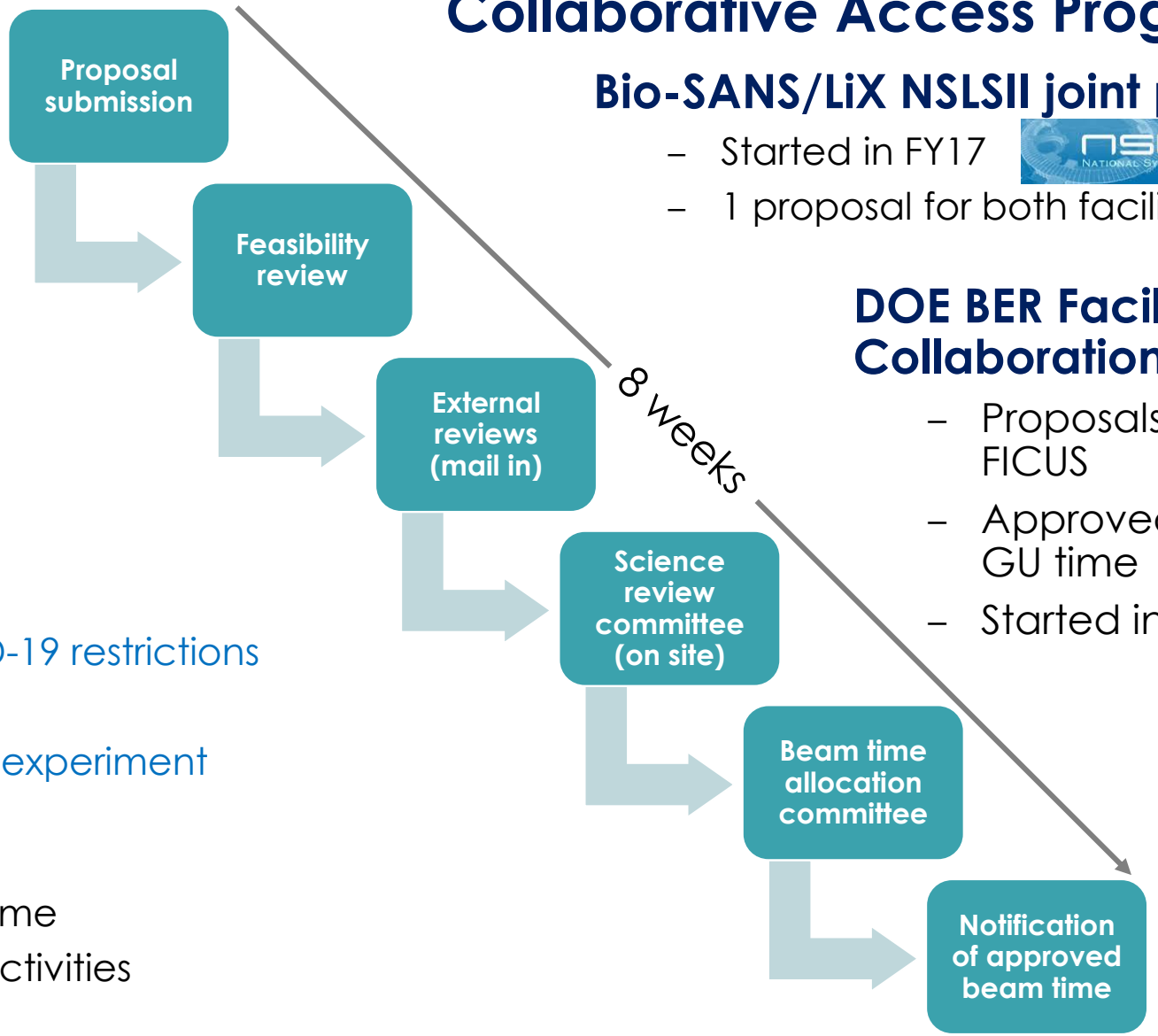
- 1 day experiment
- Internally reviewed

Remote Access

- Established during COVID-19 restrictions
- GU and POP mechanism
- User can control and run experiment

Discretionary time

- 25% of available beam time
- Program development activities



Collaborative Access Programs

Bio-SANS/LiX NSLSII joint program

- Started in FY17
- 1 proposal for both facilities

DOE BER Facilities Integrating Collaborations for User Science

- Proposals submitted through FICUS
- Approved proposals allocated GU time
- Started in FY21

FICUS Facilities Integrating Collaborations for User Science

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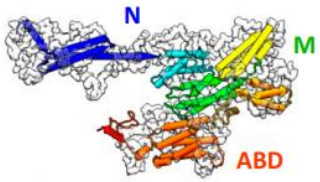
BER Structural Biology Resources

Resources for users

- Neutrons.ornl.gov
  - News and events (https://neutrons.ornl.gov/full-calendar)
  - ORNL Neutron Times (https://mailchi.mp/ornl/neutrontimes)
- SNS-HFIR User Group (SHUG)
  - https://elist.ornl.gov/mailman/listinfo/shug
- Center for Structural Molecular Biology
  - https://www.ornl.gov/facility/csmb
- DOE BER Structural Biology Portal
  - https://berstructuralbioportal.org/

Enabling **USER** science by addressing key challenges in understanding complex biological systems

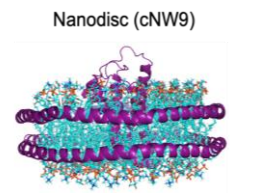
Macromolecules and Their Assemblies



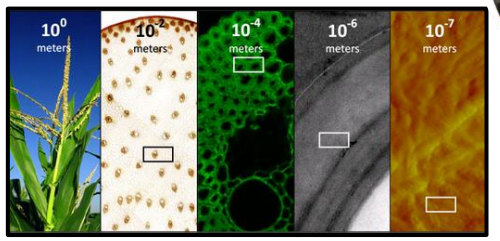
$^{d}\alpha$-catenin in $^{d}A^{h}B^{h}E$ complex

Bio-membranes

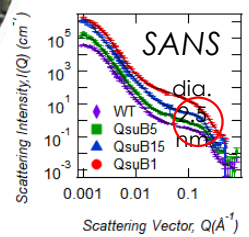
Biomimetic/Bioinspired Systems

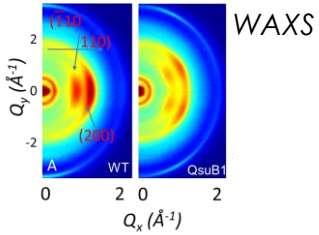


Lipid ratio = 3:2 DMPC:DMPG



Complex Systems





Biomass and Biofuels

Facility Acknowledgment Statement

- A portion of neutron scattering research presented as examples in this introduction used resources at the High Flux Isotope Reactor or Spallation Neutron Source, DOE Office of Science User Facilities, operated by the Oak Ridge National Laboratory.
- The Bio-SANS of the Center for Structural Molecular Biology at the High Flux Isotope Reactor is supported by the Office of Biological and Environmental Research of the U.S. DOE.