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ORNL Second Target Station Project: Biological & Environmental Science Workshop

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Second Target Station Project
Biological and Environmental Systems Science Directorate

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ABSTRACT

Recent advances in neutron sources and instrumentation have opened up many new opportunities for the application of neutron scattering techniques in the biological and environmental sciences. Neutrons enable studies of the structure and dynamics of biological and environmental samples with a particular sensitivity to light elements, such as hydrogen, which is a key component of biological and environmental samples. Studies using neutrons are complementary to X-rays and have the unique advantage of being non-destructive and highly-penetrating. Oak Ridge National Laboratory's upcoming Spallation Neutron Source (SNS) Second Target Station (STS) will provide high brightness cold neutron sources that significantly advance the scientific capabilities of neutron scattering instruments. The STS will advance our understanding of biological and environmental processes across spatial and temporal scales. The capabilities will enhance our ability to discover, design, and develop new materials essential for advanced sustainable technologies to address society's most pressing needs. This report summarizes the discussions and recommendations from a joint workshop held by the STS Project and the Biological and Environmental Systems Science Directorate (BESSD) in June 2022. The purpose of the workshop was to explore science opportunities and capabilities related to biological and environmental systems that could be incorporated into both current and future STS instrument designs, as well as additional instruments at the SNS First Target Station (FTS) and High Flux Isotope Reactor (HFIR). With six breakout sessions, each with two invited plenary speakers from other institutions, the participants discussed a wide range of topics relevant to biological and environmental research. Based on the input from participants, a number of recommendations on instrumentation, sample environments, complementary multi-modal methods, data processing and analysis and sample deuteration are provided in the report. The participants also identified science opportunities that are emerging from the planned instrument capabilities at STS. Selected recommendations and science opportunities are listed in the Executive Summary.

1. EXECUTIVE SUMMARY

The Second Target Station (STS) Project and the Biological and Environmental Systems Science Directorate (BESSD) organized a joint workshop in June 2022 to explore science opportunities and capabilities related to biological and environmental systems that could be incorporated into both current and future STS instrument designs, as well as additional instruments at the Spallation Neutron Source (SNS) First Target Station (FTS) and High Flux Isotope Reactor (HFIR).

The three neutron sources at ORNL will provide a suite of instruments with complementary characteristics for neutron scattering studies. Among them, the STS will be a new, high brightness source optimized for cold neutron production, ideal for biological and environmental research. The high brightness cold neutron beams will allow experiments to be performed under a wide range of conditions using smaller sample sizes and also enable time-resolved measurements of faster kinetic processes. During the conceptualization of the STS instruments, many biological and environmental topics were included as scientific drivers by almost all instrument proposal teams. After the selection of eight initial STS instruments in July 2021, the STS Project and BESSD organized this joint workshop to take a more comprehensive look at capabilities across the selected instruments. The goal of this workshop was to identify any missing science themes that could inform the instrument design in the next phase of the design process, and to identify science opportunities that the next wave of STS instruments could address. In addition, this workshop also identified capability and capacity gaps that might create an opportunity for DOE Office of Science – Biological and Environmental Research (BER)-funded instruments to be built at STS.

The workshop had a total of six breakout sessions, each with two invited plenary speakers from other institutions, followed by discussions among participants. The session topics were (1) Soil, rhizosphere processes & root water dynamics; (2) Biological systems, synthetic biology, and enzyme engineering; (3) Plant-microbe interactions and global change biology: Methods complementary to neutrons; (4) Biopreparedness; (5) Next generation bioproducts, biomaterials and bioenergy; and (6) Geochemistry: Nanoscale structure of porous materials.

Based on the workshop input, a number of recommendations are summarized in the report. First, neutron imaging, which has proven very useful in exploring flow-through porous media, soil-root and root-water interaction research, can be widely applied to many other research topics with further developments such as additional sample environment configurations, improved detector resolution, and faster data collection for larger samples. Many of those requirements are not unique to neutron imaging instruments. In addition to capability gaps that the STS instruments are filling, it was noted by the participants that increased capacity is needed as the user community expands. For example, additional beamline capacity, such as neutron imaging, small-angle neutron scattering (SANS) instruments and protein crystal diffractometers at STS that are dedicated to biological and environmental research would help to meet the demand from the research community and build a sustainable user community. The development of multi-modal measurements with sophisticated samples for in-situ/in-vivo experiments at neutron scattering instruments was also a general consensus in the workshop discussion. With much higher data rates anticipated from STS instruments, new data processing, reduction and analysis methods and tools become imperative to make instrument operations more efficient and user experiments more productive. Specifically, analysis tools that take advantage of artificial intelligence and machine learning methods along with supercomputing should be developed in tandem with instrumentations to fully realize the potential of the new facilities at STS. Last but not the least is the development of sample deuteration capabilities to enhance neutron contrast. The manipulation of neutron contrast with deuteration represents one of the greatest advantages in neutron scattering. The current lack of advanced deuteration capabilities in higher eukaryotes and for cell-free protein production is a major barrier for many high impact neutron experiments.

The workshop participants also contributed many current and emerging scientific topics that could serve as exemplary science drivers for current instruments and their auxiliary equipment design as well as future instrument concept development. They are listed as part of recommendations in Section 3.

2. INTRODUCTION

Discovery science drives innovation and underpins the technological advances that will solve some of society's most challenging issues, including climate change, clean renewable energy technologies, better medicines, safe potable water, and address aging infrastructures, including transportation. Many of these advances will result from basic research to advance our understanding of biological and environmental processes and materials. Oak Ridge National Laboratory's neutron sources provide cutting-edge scientific tools to probe the structure and dynamics of matter in unique ways. This insight is fundamental to advancing our understanding of biogeochemical processes across spatial and temporal scales and their impact on the Earth's climate using high resolution earth system models and our ability to discover, design, and develop new materials essential for advanced sustainable technologies to address society's most pressing needs.

The Spallation Neutron Source Second Target Station (STS) will be a new, high brightness source optimized for production of cold neutrons. It will provide transformational new capabilities that complement the strengths of the current ORNL neutron sources. The high brightness cold neutrons will enable time-resolved measurements of kinetic processes. The intense neutron beams will allow study of smaller samples of biological and environmental samples or materials under a wide range of conditions. The STS pulse rate of 15 Hz enables measurement of structure and dynamics across a broad range of length scales.

The initial suite of eight neutron instruments that will be built as part of the STS Project has been selected (https://neutrons.ornl.gov/sts/sts-instrument-systems). While many instrument proposals included BER (the U.S. Department of Energy's, Office of Science, Biological and Environmental Research program, https://www.energy.gov/science/ber/biological-and-environmental-research) science in their science cases, it is beneficial for a more comprehensive look across the selected instruments to identify any missing science themes that could modify instrument designs and identify science opportunities that the next wave of STS instruments could address.

The goal of this workshop is to identify opportunities for BER science on the current suite of selected instruments and identify capability gaps that might create an opportunity for BER-funded instruments to be built at the Second Target Station. This report is a summary of the workshop. It describes many BER science themes that can be pursued on the 8 selected instruments and additional special requirements that need to be considered in the early design of these instruments, for example, the application of simultaneous or sequential multi-modal techniques, among many other comments and suggestions. The report also describes capability gaps in the ORNL neutron instrumentation suite that could be addressed by new or improved instruments at any of the three ORNL neutron sources (HFIR, SNS-FTS and STS).

Workshop participants were encouraged to consider the following questions in their discussions:

- *Identifying current methods or approaches in biological and environmental science where neutrons could provide complementary information.*

- *Are there unique requirements needed to support BER science that should be considered in the current 8 STS instrument designs? Are there synergistic or multimodal capabilities that should be incorporated into neutron beamlines?*
- *Are there any special requirements for data analysis and reduction that should be considered for the 8 selected instruments?*
- *What are the emerging and projected biological and environmental science grand challenges where neutrons can provide unique information?*
- *What are current methods, systems or approaches in biological and environmental science where neutrons can provide complementary information?*
- *What science-driven capabilities are needed beyond those of the current ORNL instrument suite and the 8 initial STS instruments?*

The workshop was held virtually on three afternoons on June 14, 21, and 28, 2022. The agenda and the list of speakers, session moderators and all workshop participants can be found in the appendix.

This report is organized by six topics corresponding to the six sessions starting with outlines of the invited plenary talks followed by summaries of the discussions in the breakout sessions. The workshop agenda and complete list of participants are attached as appendices.

3. RECOMMENDATIONS BASED ON THE WORKSHOP INPUT

Capability gaps in the ORNL neutron instrumentation suite that could be addressed by new or improved instruments at the High Flux Isotope Reactor, the Spallation Neutron Source First Target Station, and the STS were discussed during the workshop. Here are recommendations based on the workshop input that are especially relevant to the science communities working on biological and environmental research.

3.1 Neutron Imaging Capabilities

Neutron imaging (NI) is a direct imaging technique based on selective absorption of neutrons by different atomic species and enables the characterization of samples across wide spatial and temporal scales. It combines the high penetrating power of neutrons with intuitive results through 2D images and tomographic visualization of samples. Many discussions in the workshop identified related research opportunities. Neutron imaging has helped provide novel insights into soil-root interactions, root water uptake and the dynamics of water availability and transport. However, we have a limited understanding of the relationships between root traits, root exudates, rhizosphere biology, soil texture and physicochemical characteristics, and how roots develop and interact with immobile bound or mobile soil water content. These research topics are particularly important in the context of plant drought stress under a changing climate. The improved and new development of NI instruments with high brightness can contribute significantly by allowing much faster data collection times, which are also advantageous for 3D tomographic image reconstruction. In addition, higher image resolution can be achieved by incorporating dark field imaging and time-of-flight (TOF) techniques.

One key objective for future advances in NI is to expand capabilities to enable research into environmental processes that are not as easily assessed, such as those related to carbon, nutrient or contaminant transport, exchange, or transformations. Chemical transformations of soil organic matter, mineral weathering, and plant-microbe assemblages are driven by processes at molecular scales, but manifest at much larger scales that are accessible to neutron imaging. The desired improvements include larger fields of view, faster data collection, and better detector resolution. Multimodal capabilities to measure complementary parameters in addition to water distribution and dynamics components could provide additional structural information, such as pore structure, interconnectivity, and soil characteristics. Small-angle neutron scattering, diffraction, X-ray computed tomography (CT), X-ray absorption spectroscopy or other multi-modal techniques may help address these research questions.

Sample environment needs for in-situ experiments with plants include a growth chamber that provides the environmental controls necessary for maintaining, preparing, or propagating samples. Similarly, pre and post access to a – 80 °C freezer onsite would enable molecular biology measurements such as genetic sequencing of biological material. In addition, specific studies require precise control of environmental conditions at the beamline during the course of the experiments, including light levels, temperature (including freezing/combustion), relative humidity, CO_2, and gas exchange, containment, collection. Further improvements in instrument hardware, e.g., detector resolution, neutron flux and overall shorter data acquisition times, will translate directly to future scientific advancements. In addition, multimodal techniques, such as other neutron scattering techniques, X-ray CT, spectroscopy, etc., would provide synergistic research capabilities.

3.2 Needs for additional capacity and high-throughput instruments

The first eight instruments selected for SNS STS are filling many capability gaps in the existing U.S. neutron scattering facilities. The combinations of instruments at each of the three neutron sources (SNS-FTS, STS, and HFIR) at ORNL provide a complete set of neutron tools for researchers to cover a wide range of time and length scales to investigate structure and dynamics. With the enhanced brightness of the STS source, sample throughput is expected to be much higher than possible at the SNS FTS and HFIR instruments. However, the discussions in the workshop pointed to a strong need for additional capacity and higher throughput as the demand for neutron beam time is expected to grow as the user community expands. Currently many beam time proposals (about 2/3) cannot obtain requested beam time due to capacity constraints. Additional capacity is expected to increase the number of experiments and will help expand the user community.

For example, SANS instruments with specific mission definitions instead of general-purpose instruments will be beneficial for different research topics between biology and other material sciences. A higher flux SANS instrument that can quickly obtain data in a limited Q range with simpler instrument setup, potentially with lower cost, would complement the abilities of CENTAUR at STS and allow high throughput characterization of biological samples such as protein complex formation, ligand binding, intrinsically disordered proteins, and other kinetics.

In addition, a dedicated single crystal protein diffractometer is needed for biomacromolecular studies. This type of instrument enabled neutron diffraction studies resulting in significant contributions to fundamental SARS-CoV-2 research during the recent COVID-19 pandemic. The current capacity as well capability limit constrains such research relating to biopreparedness.

3.3 Multi-modal measurements with more sophisticated sample environments

Many complex systems will benefit from multi-modal measurements that complement neutron scattering and direct imaging techniques. Ideally, multi-modal measurements are carried out under the same conditions and allows collection of complementary data simultaneously with the on-going neutron experiment. The incorporation of multi-modal capabilities requires development of more sophisticated sample environment equipment that accommodates not only characterization by different techniques, but also provides proper conditions needed such as temperature, relative humidity, lighting or more. A specific recommendation is a combined light microscopy and USANS instrument for the study of hierarchical and complex systems. This capability would provide new insights into multicomponent systems with different types of contrast (light vs. neutron). Another example of an attractive multi-modal measurement is to combine high-flux X-ray instruments (e.g., with metal jet X-ray generator) with the high flux neutron instruments at STS. Such a capability would improve the accessible timescales enabled by faster X-ray measurement and accelerate research greatly.

The development of versatile sample environments was mentioned frequently in many discussions of the workshop. As the systems of interest varies greatly, a wide range of sample environments are needed. One interesting example is a sample environment to sustain artificial tissues or organs (organoids), which have attracted significant interest recently. The non-destructive nature of neutrons could offer many benefits for studies in this area in the near future. Other sample environment suggestions include the incorporation of pump-probe techniques for observing dynamical protein configuration changes in situ, and environmentally controlled setup in which CO_2 and O_2 levels and temperature can be manipulated. Configurations that allow for the observation of enzyme reaction cascades with capabilities for titration of substrates and for templating or polymer patterning in vitro are also desirable. For the geochemistry community, sample environments that support in-situ characterization studies under extreme conditions, e.g., high pressure cells providing geologically relevant pressures, need to be developed.

One particular recommendation was the development of a universal sample holder that is compatible and exchangeable among different beam lines, light sources and other instruments. This will facilitate the collection of multiple data types across labs and institutions with improved consistency. Standardization of sample environments and holders across beamlines and user facilities, where possible, is expected to bring significant benefits to multi-modal and multi-probe measurements. Thus, the integration and analysis of multi-modal data from different techniques needs further development, which also is underscored in the data analysis recommendations below.

3.4 New capabilities for data processing and analysis

With advances in neutron instrumentation, storage, processing, and analyses of larger and more complex data sets become a barrier for achieving the expected, higher level of productivity for many neutron experiments. For example, real-time image analysis during the experiment is highly desirable so that adjustments can be made to samples or instrument parameters to optimize the next measurement. New capabilities for data handling, processing and analysis are needed. Artificial intelligence (AI), machine learning (ML) methods and supercomputing are emerging rapidly and should be adopted more widely for the future instruments. Many aspects of experimental design, real-time data processing/analysis, instrument configuration optimization and even the experiment steering could be coupled to AI/ML and supercomputing capabilities to best utilize neutron instruments. For example, AI/ML could be used during data processing to enable lower signal-to-noise ratios in data sets. This process would shorten typical neutron data collections and help reduce the number of background runs which use significant beam time on a SANS measurement.

Furthermore, analysis methods and tools that can combine data from multi-modal measurements or other techniques need to be developed. For example, combining static high resolution cryo-EM data with SANS/SAXS data can reveal both detailed structures and configurations of a biomolecular system in solution providing more biologically relevant information. The development of user-friendly computational tools that don’t require specialized computational expertise is needed to bridge those techniques. These approaches will accelerate and complement the continued need for expert beam-line advice and interpretation.

3.5 Deuteration capabilities for sample preparation

The large difference in neutron scattering length of hydrogen isotopes protium and deuterium provides a beneficial and unique tool for understanding many biological and environmental samples, which are abundant in hydrogen. Deuteration is an essential component of sample preparation for successful neutron scattering experiments, which was greatly emphasized by many participants. For studies on proteins, there is a need to develop more advanced, targeted deuterium labeling strategies in higher eukaryotes and cell-free expression systems. In addition, specific deuteration of biomolecules such as lipids or RNA/DNA, also needs further development as components and ultimately within larger more complex systems.

3.6 Science opportunities

Examples of current and emerging science opportunities identified in the workshop:

- Plant drought stress is highly relevant considering the current climate trajectory. Water and nutrient availability are dependent on continued contact between soil and roots, yet during drought the hydraulic contact across the rhizosphere may be lost due to development of a soil-root gap or physical shearing of fine root, root hair or fungal hyphal structures, processes that are largely unquantified.

- The interactions between root traits, root exudates, rhizosphere biology, soil texture and physicochemical characteristics, how they develop and how they interact with immobile bound or mobile soil water content are largely unknown.

- The role of plant-microbe assemblages in mineral weathering and carbon sequestration in the context of soil mesostructure and soil aggregate organo-mineral complexes.

- The importance of membrane protein interfaces and perturbation by solvents or other stressors in biomembranes.

- The structure and function of intrinsically disordered proteins alone and in complexes.

- There are many knowledge gaps with respect to the fundamental behavior of biological systems in different environments. These insights are needed to improve synthetic biology approaches. Examples include the formation of enzyme complexes in different environments, the dynamics and mechanical properties of biological membranes, flexibility, and disorder in biomacromolecular complexes and biomaterials including biopolymers.

- Organisms in extreme, yet biologically relevant sample conditions (e.g., high temperature and pressure).

- Multiscale systems in biological research, which aims to connect molecular, cellular, tissue and organism level structure. Such multiscale studies may apply to advanced sustainable environmental systems (e.g., plants, roots), as well as to biomedical applications, such as artificial tissue and organ development.

- How microorganisms are distributed in the soil, rhizosphere, and along the plant root in intact biosystems. In particular, there was interest in identifying and even tracking different bacterial species simultaneously within these complex communities over time.

- The need for identifying a few key model systems that can be manipulated and labeled to enable multi-modal imaging by neutrons and other selected imaging modalities.

- Plastics and biopolymer upcycling and reuse by biology. For example, at the enzyme level, enzyme supercharging as well as multimodal visualization of cellulase activity on cellulose surfaces can provide valuable information on upcycling by biological methods. Such studies could inform development and engineering of chemical processes for upcycling of lignocellulosic and plastic-based waste materials to value-added products such as biofuels.

- Geochemistry: Tracking of interfacial reactions in real time to separate the roles of kinetic versus thermodynamic behavior. Constrain elementary mechanisms of how a fluid-solid interface responds near or far from equilibrium. Translate molecular-scale description of interfaces to thermodynamic or kinetic quantities for the purpose of linking mesoscale to macroscopic models.

- Fluid and mineral behavior at extreme conditions is a wide-open area of fundamental research in earth science. Example questions related to phase transition in extreme conditions: what triggers the phase change? What is the role of defects or hydrogen? How are the chemical and mechanical processes coupled during phase transition?

4. REPORT OF PRESENTATIONS AND DISCUSSIONS

4.1 Topic 1: Soil, rhizosphere processes & root water dynamics

The first topic, on Neutron Imaging (NI) of soil rhizosphere processes, focused on assessing the relatively large-scale processes (ranging from µm to m scales) occurring within soils and living plants *in situ*, with a focus on soil-root interactions and root water uptake and transport through the soil-plant-atmosphere continuum. The basis for this topic has been to explore novel insights into mechanistic physical processes belowground and within plant xylem in order to improve hydrological modeling and develop new understanding of the dynamics in water availability and transport under drying or wetting conditions. The large neutron cross-section of hydrogen and the availability of an excellent surrogate in deuterium has provided the contrast needed to readily address research questions related to water dynamics in geosciences using neutrons. NI research has validated prior indirect measurements of soil water infiltration, transport, and root water extraction, but also provided novel mechanistic insights related to root-rhizosphere-soil water dynamics. These results can be translated into a modeling framework to test and refine current assumptions used in hydrological modeling that simulates transport of water and soil-plant responses to drying conditions. One key objective for future advances in NI is to expand capabilities to enable research discovery of processes that are not as easily assessed, such as those related to carbon, nutrient or contaminant transport, exchanges or transformations.

Brief summary of the plenary talks

The first talk on this topic was presented by Dr. Christian Tötzke, a professor in the Institute of Environmental Sciences and Geography at University of Potsdam, Germany. Dr. Tötzke is an expert in three-dimensional neutron computed tomography (NCT) imaging of root water uptake, redistribution and translocation *in situ* and has also explored soil-root water imaging using magnetic resonance and synchrotron X-ray imaging. His talk highlighted the ability to use 2D or 3D imaging to assess root structural architecture, traits, and root growth. 2D imaging has long been used for assessing infiltration of water and root water uptake and redistribution. In this talk, Dr. Tötzke presented compelling images and movies showing some of the first time-resolved 3D imaging of root water uptake in soil. This was achieved using deuterium oxide as a contrast agent and ultra-fast NCT, a technique that his group has been pioneering. Using wild-type and mutant maize that vary in root hair density, he also described the ability to assess differences in root characteristics *in situ*, including their response to drying conditions. These preliminary results revealed that the roots shrank during soil drying, thereby developing a gap, but that the root hairs adhered to the soil particles, thereby maintaining root-soil hydraulic contact that could provide a continued path for water flow into the plant. The use of dual mode NCT and X-ray CT provided the complementary data to achieve this, with the paired measurements of root distribution, water flow and soil structure necessary to explore this dynamic research.

The second talk was led by Dr. Mutez Ali Ahmed, an assistant professor in the Department of Land, Air and Water Resources at the University of California, Davis. Dr. Mutez has expertise in two-dimensional neutron radiography of plant systems, hydraulic redistribution of water within the soil by roots, and plant responses to drought. In context of this latter point, his talk focused on the question of which roots take up water, and where that water is extracted from in the soil profile. Using maize, results indicated root types, root traits and different root xylem maturation rates all have impacts on water uptake rates and locations, independent of abundance of water availability. In addition, Dr. Mutez showed how uptake by roots in wet soil could be translocated and used to support root growth into dry soil. A modeling framework was presented that assessed relative contributions of diffusive and convective root water uptake, and the different mechanistic limitations in water uptake due to xylem hydraulic capacity in wet soils or soil water transport and radial root uptake in dry soils. Dr. Mutez also presented novel neutron imaging of gap formation around the root system during drought. In this case, rather than the mechanism of root hairs pulling soil particles towards them during drying, mucilage previously exuded from the root adhered the root to one side of the soil gap in the rhizosphere during drying, thereby keeping part of the root against the soil and thus root-soil hydraulic contact. Interestingly, the mucilage was shown to have hydrophobic properties, that varied during drying and wetting cycles, and which may play a role in root water availability.

Discussions on charge questions

In the break-out session, the talks were further discussed in addition to what other current and future emerging science questions related to soil-rhizosphere-plant processes that might be addressed with NI. Plant drought stress was a key topic, and highly relevant considering the current climate trajectory. Water availability in the rhizosphere may become limited by gaps between soil and roots, resulting in loss of hydraulic contact due to the gap itself or physical shearing of fine root, root hair or fungal hyphal structures. But these processes have not been readily imaged or quantified. Similarly, there is little direct understanding of the interactions between root traits, root exudates, rhizosphere biology, soil texture and physicochemical characteristics, how they develop and how they interact with immobile bound or mobile soil water content. These relationships and interactions change over time and will regulate soil water and dissolved solute availability for root uptake but uncovering the pore-level mechanisms has been limited due to a lack of suitable techniques to study plant root uptake in vivo.

Symbiotic root-fungal relationships are central to root resource extraction from rhizosphere and soil pools. Such root-microbe interactions are highly complex, and include symbiotic, mutualistic, and competitive nutrient, carbon and water exchanges, but mechanisms and quantification of these exchanges to changing conditions is not well understood. The processes occurring at the root-rhizosphere interface are not well known, including regulation of root resource extraction by root membrane bound nutrient transporters or aquaporins and their response in real time to internal or external drivers. Advances in NI could provide new understanding of the role of soil fungi on soil hydraulic properties, soil water content, root nutrient or water uptake, biogeochemical cycling and soil carbon storage.

New insights into mechanistic, *in situ* soil chemical transformations and soil organic matter dynamics were also discussed - processes that often occur at the sub-μm scale below typical NI resolution but manifest at much larger scales. There is an interest in additional insights into the role of plant-microbe assemblages in mineral weathering and carbon sequestration in the context of soil mesostructure and soil aggregate organo-mineral complexes. New research is needed to quantify and track the dynamics of soil carbon, particulate and mineral interactions with soil pore structure and soil surfaces, including assessment of how soil aggregation and pore properties impact soil organic matter storage. Physical processes such as freeze/thaw or combustion dynamics within soils provide another element to research needs. As well as dynamics, transformation, transport and fate of contaminants or microplastics, especially in agricultural soils. Indeed, transport processes was another main topic of discussion, particularly in the context of transport of particulates, solutes or colloids in porous media or soils. While NI can readily track water dynamics, the group realized the need for multimodal capabilities to track other components, and to provide additional structural information of the porous media, including pore structure, interconnectivity and characteristics. Similarly, there is a need to link plant function to plant traits, both above and belowground, and dynamics in their characteristics and relationships to resource extraction or plant damage in response to external stressors. Advances in NI, small-angle neutron scattering, diffraction or other neutron or multi-modal techniques may help address these and other pressing research questions in geosciences.

Then we considered what type of sample environments, techniques and analyses capabilities are needed in current or future beamlines to enable testing our research questions. As soil-rhizosphere-plant systems are living and dynamic, there are unique needs for NI experiments. Having a growth chamber onsite with environmental controls is necessary for maintaining, preparing or propagating samples. Similarly, pre and post access to a – 80 °C freezer onsite would enable molecular biology measurements such as genetic sequencing of biological material. In the beamline, the sample environment must be controllable for specific studies (e.g., controlled exposure to light, temperature enabling both freezing and combustion, relative humidity, CO_2, and gas exchange/containment/collection. Environmental conditions within the beamline must be precisely monitored. Dynamic processes also require fast collection of data through large samples, ideally in 3D. AI and supercomputing were mentioned for their utility in real time analysis/reconstruction, which could provide fast results and direction to the current or future experiments. Data management and storage are also key features. For the beamline itself, we discussed the idea of variable depth of beam focus into a sample. Certainly, we can get at this with NCT, but can this be achieved faster in 2D? Another component discussed was detector resolution – could this be variable and changed in near real time, such as turning a microscope lens, e.g., changing resolution from 50 μm to 1 μm or higher? As mentioned earlier, the use of multimodal capabilities at an imaging beamline, such as other neutron scattering techniques, X-ray CT, isotopes (stable and radioactive), enzymes, fluorescence, etc., would provide synergistic research capacity.

While ORNL currently has the reactor-based HFIR CG1D Imaging beamline and the accelerator-based SNS SNAP beamline that can be used for energy selective imaging, we expect much greater capabilities and improved resolution with the planned HFIR upgrade, the new SNS VENUS Imaging beamline, and the planned future CUPI2D Imaging beamline. While the new and expanded capabilities of these beamlines are expected to facilitate novel and cutting-edge research, simultaneous or sequential multimodal imaging will be required for next-level research questions including those that span across a broader range of time and spatial scales.

4.2 Topic 2: Biological systems, synthetic biology and enzyme engineering

Brief summary of the plenary talks

The first of the plenary talks was delivered by Jonathan D. Nickels who is currently an Assistant Professor at the University of Cincinnati. The title of his talk was, Structure and Solvent Partition Coefficients for Living Cell Membranes and Model Membrane Mimics. Dr. Nickels gave a brief overview of collaborative work that spans several years between his group and ORNL researchers involved with neutron sciences, computational biology, and biosciences. One particular project that was emphasized is focused on understanding physio-chemical changes that occur in biological membranes upon exposure to advanced biofuels such and n-butanol. In collaboration with ORNL, unique capabilities have been developed to study biomembrane structure in living bacterial cells using small-angle neutron scattering techniques (SANS). Dr. Nickels provided a perspective on what he considers from his experience to be the most useful capabilities at the STS for biological systems science. This included a brief discussion on instruments necessary for structural studies including SANS and reflectometry as well as dynamic studies and possible new modes of imaging to better capture dynamic phenomena in biomaterials, proteins, and lipid membranes. The remainder of the presentation provided background on the power of H/D isotopic labeling and varying contrast conditions which provides a powerful tool to study biological systems including biological membranes using SANS. Experimental data was presented from SANS experiments that showed how neutrons could be applied to determine membrane thickness in model and living membrane systems as well as lateral structures in the form of lipid rafts. New data from the Nickels lab is pointing toward the possibility that solvents such as ethanol perturb the lateral organization of biomembranes and affect lipid raft structure. This could potentially be an early failure mode in membrane homeostasis while under solvent stress. Ongoing collaborative work with ORNL was summarized and it was an overall excellent presentation.

The following plenary talk was presented by Dr. Yannick Bomble, Group Research Manager in the Biological Science Group at the Renewable Energy Laboratory, Golden, CO. Dr. Bomble's group is interested in topics including active research areas related to biomass deconstruction by thermophilic microbes, plant cell wall biosynthesis, and cell-free biocatalysis using engineered systems. Several research highlights were presented that included examples of how neutron scattering experiments were or could be used to resolve differences or changes in structure for several enzymes related to the topics mentioned above. Much progress has been made by the group and their collaborators on characterizing extremely thermostable cellulases (namely CelA) produced by *Caldicellulosiruptor bescii*. Molecular dynamics simulations and SANS have been applied to better understand the glycosylation states of these enzymes which could eventually enable high-level heterologous expression of fully active enzyme complexes for improved biomass solubilization. Other neutron-based studies were able to assist in determining the structure of CelA when in solution or bound to its insoluble substrate. Furthermore, structure-based studies were discussed that involved enzymes responsible for xyloglucan synthesis in plant cell walls and optimizing peptide linker structures and length for optimal activity of immobilized enzymes applied to cell-free systems for biocatalysis. Dr. Bomble's group is a user of the neutron facilities at ORNL and is excited to collaborate on multiple projects in the future.

Discussions on charge questions

The discussions in the breakout session were guided by the charge questions and focused on several topics related to emerging science areas, data analysis and instrument capabilities.

The effect of solvent perturbations and impacts on the dynamics and activities of enzymes and proteins with and without immobilization was discussed. The participants highlighted the importance of membrane protein interfaces and perturbation by solvents or other stressors. Insights into fundamental behavior of biological systems in different environments is needed to improve approaches to synthetic biology. Examples include the formation of enzyme complexes in different environments, the dynamics and mechanical properties of biological membranes, flexibility and disorder in biomacromolecular complexes and biomaterials including biopolymers. Neutron spin echo experiments can provide particularly useful information on the dynamics complementing computational approaches, such as molecular dynamics.

Experiments with living organisms in extreme, yet biologically relevant sample conditions (e.g. high temperature and pressure) could help close the gap between model systems and biological processes in natural or engineered environments. For example, studies on the interaction between cellulose-degrading microorganisms and the surface of a cellulose microfibril could provide molecular scale insights into the hydrodynamic environment of this interface. Additional use cases discussed were bioelectronics, membraneless organelles, 3D structural organization of genomes, biological redox enzymes (e.g. N_2 fixation), and the study of quantum effects that may occur in biological systems. The importance of multiscale systems biology research, which aims to connect molecular, cellular, tissue and organism level structure and organ development was highlighted. Such multiscale studies may apply to biomedical applications, such as artificial tissue and organ development as well as studies on advanced sustainable energy systems.

New capabilities for data analysis needed to process large and complex data sets were discussed. Machine Learning (ML) and supercomputing are emerging rapidly and could be applied to help in the experimental design of neutron scattering experiments, real-time data analysis and processing of large data sets. Examples include approaches to predict scattering profiles from short measurements, steering of neutron experiments using real-time data analysis using advanced computing resources. Integration of STS data acquisition systems with ORNL supercomputing resources could enable supercomputer-driven steering of neutron experiments in real time. In addition, multimodal analysis approaches that integrate and leverage insights from multiple analysis methods (simultaneously) could further help with data interpretation. For example, combining cryo-EM and SANS/SAXS can reveal how the behavior of a biomolecular system in solution varies from the CryoEM environment resulting in a more biologically relevant description of the system. The integration of computation methods, such as molecular dynamics and QM/MM calculations, with neutron crystallography can offer unprecedented insights into enzyme reaction mechanisms.

The importance of deuteration for the study of biological structure and dynamics was emphasized. The is a need to develop more advanced, specific deuterium labeling strategies in higher eukaryotes as well as cell-free expression systems. There is a need to better understand the effect of deuteration on biological structure and dynamics, both experimentally and theoretically. High-resolution mass spectrometry and low-volume sampling from sample chambers would offer direct insights into molecular changes and deuterium substitution. These new approaches also call for the development of sample holders and sample environments that support multi-modal analysis techniques. In addition, to these capabilities needs high sample throughput with fast yes/no feedback enabled by higher neutron flux and wider q-range were discussed.

There is a strong need for additional sample capacity and increased throughput. This could be achieved by less expensive SANS instrument design and higher flux instruments. In addition, there is a need among the user community for instruments, such as VSANS, neutron imaging/tomography, NSE, neutron crystallography, and neutron reflectometry. Instruments that enable studies with polarized neutrons could also help increase additional capability, for example Spin Echo Modulated SANS expands SANS to measure micron-sized structure, and can be particularly helpful for addressing specific science questions.

4.3 Topic 3: Plant-microbe interactions and global change biology: Methods complementary to neutrons

The interactions of plants and microbes with each other and the environment underpin many of the processes of interest to DOE. For example, the microbiome of plants influences plant health and productivity and can help modulate responses to environmental stresses. Likewise, plants and microbes play a key role in carbon and nitrogen cycling. A better understanding of how plants and microbes interact with each other can offer insights into ecosystem resiliency and sustainability, as well as identify new strategies for bioenergy production and bioremediation.

Imaging plant-microbe interactions, however, represents a temporal and spatial scaling challenge. For example, the uptake and exchange of metabolites and macromolecules between plants and microbes can occur at very short time and length scales, whereas microbial recruitment, microbiome assembly, and plant growth typically occur over longer time and length scales. For these reasons, the use of multiple imaging modalities to acquire complementary information about the same biosystem provides an attractive approach to overcoming this challenge. As such, this workshop topic focused on the use of imaging methods that are complementary to neutron imaging to investigate plant-microbe interactions.

The first speaker for this session was Dr. Kenneth Kemner, the leader of the Molecular Environmental Science group at Argonne National Laboratory. His presentation focused on the use of synchrotron x-ray radiation to study plant-microbe biosystems. X-ray fluorescence imaging is used to map the distribution of elements and chemical species within biological samples. In one example, Dr. Kemner described recent efforts to image the distribution of bacteria in a soil aggregate. To label the bacteria for imaging, they utilized quantum dots which were taken up by the microbes and provided contrast for x-ray fluorescence imaging. This approach allowed for a 3D reconstruction of a soil aggregate showing voids in the soil particle that were occupied by labeled microbes.

The second plenary speaker in this session was Dr. Marcus Cicerone, a professor in the Department of Chemistry and Biochemistry at Georgia Institute of Technology. His seminar focused on the use of coherent Raman scattering microscopy which allows the identification of different molecules based on their vibrational signatures. This method is often used to identify biomolecules, such as lipids or proteins, in fixed or live samples without the use of exogenous labels or stains. Dr. Cicerone briefly described his efforts to develop a microscope based on coherent Raman scattering and coherence-gated detection that will be used to understand metabolic interactions between plants and nitrogen-fixing bacteria.

Following the plenary talks, the discussion began with some brainstorming on current or emerging science areas where neutrons and complementary methods can provide insights. Consistent with the theme of plant-microbe interactions, there was considerable interest in understanding how microorganisms are distributed in the soil, rhizosphere, and along the plant root in intact biosystems. In particular, there was interest in identifying and even tracking different bacterial species simultaneously within these complex communities over time. Current efforts to track multiple species within a complex community typically requires destructive sampling or the use of genetically modified bacterial strains. It was suggested that the use of boron nanoparticles or isotope labeling of specific microbes would enable imaging by both x-ray fluorescence and neutron imaging. Likewise, there was interest in understanding how changes in soil porosity over time influenced the distribution of microbes and there was a good discussion about using multi-modal methods to correlate water uptake by the plant to the distribution of elements and microorganisms in intact biosystems. One recommendation from the group was to identify a few key model systems that can be manipulated and labeled to enable multi-modal imaging by neutrons and other selected imaging modalities to demonstrate the power of correlating imaging data across time and length scales.

Related to the first topic, the group then discussed the essential requirements and capabilities that should be incorporated into the neutron beamlines. To enable imaging of spatial and temporal dynamics in living plant-microbe systems, there is a need to incorporate environmental controls, including temperature, light, and humidity, at the beamlines. There was much interest in having chemical imaging capabilities, such as Raman spectroscopy or imaging mass spectroscopy, co-located or easily accessible to the neutron beam lines to facilitate multi-modal and multi-scale measurements. Related to this, there was the recognition that development of a universal sample holder that is compatible with different beam lines, light sources, and instruments would facilitate the collection of this data across labs and institutions. Thought should also be given to the development of appropriate fiducial markers that can be utilized to align images from different time points or instruments. Finally, the group identified the need to have real-time image analyses so adjustments can be made to sample preparation or imaging parameters to optimize data quality.

4.4 Topic 4: Biopreparedness

In March 2020, at the beginning of COVID-19 pandemic, the U.S. Department of Energy (DOE) Office of Science (SC) established the National Virtual Biotechnology Laboratory (NVBL) Harnessing capabilities across all 17 DOE national laboratories, NVBL made critical advances by collectively ing DOE basic science and experimental user facilities—including x-ray and neutron sources, leadership computing facilities, nanoscale science research centers, and biological characterization laboratories to provide responses and resources for the nation's needs. This resulting research delivered the foundational expertise and capabilities necessary to meet some of the greatest scientific challenges during the pandemic. This workshop session was focused on discussions on expanding ORNL's contributions to biopreparedness efforts that enable a rapid and comprehensive response to potential future biological events and threats.

Invited presentations were given by Dr. Michelle Buchanan (DOE) and Dr. Susan Tsutakawa, (LBNL). Dr. Buchanan discussed DOE's recent NVBL efforts and potential areas for future Bio preparedness efforts. While Dr. Tsutakawa discussed the role of small angle scattering and the unique insights it can give into viral proteins in solution along with developments in protein structure prediction that will be helpful to rapidly develop structural models of proteins from new biological threats. After the two invited presentations a breakout session was held where the charge questions were considered

Discussions on charge questions

In the breakout session it was suggested that early detection of biological threats by characterizing host/receptor dynamics in solution or within a membrane coordinated with computational analysis of animal host range to predict future diseases that are naturally transmitted between animals and humans (Zoonoses). small angle Neutron scattering and dynamics measurements in solution are two techniques that were thought to be useful for this work.

The breakout session members thought that artificial intelligence (AI) is expected to play a big role in future optimization of neutron experiments. Several ideas were discussed including the use of AI to help interpret short neutron data collections, low signal to noise data collections and to help reduce the number of background runs required in a typical SANS experiment.

Sample environments to sustain artificial tissues are organs were thought to be useful in the future as significant interest is expected in this area over the next ten years. The ability of neutrons to study cells and artificial tissues without damaging them was predicted to become an increasingly important area for neutrons in biology.

The breakout session members suggested three instrument concepts that should be further developed.

- A combined light microscopy and USANS instrument for the study of hierarchical and complex systems at STS. This multimodal instrument would be able to give new insights into multicomponent systems.
- A dedicated single crystal protein diffractometer for STS. During the recent pandemic this class of instrument allowed neutrons to make a significant contribution to research on SARS-CoV-2.
- A high flux SANS instrument for STS that can quickly obtain data in a limited Q-range would complement the abilities of CENTAUR and allow for time-resolved characterization of protein complex formation, ligand binding and kinetics.

4.5 Topic 5: Next generation bioproducts, biomaterials and bioenergy

BER has a mission in the development of fundamental systems biology and biotechnology for DOE missions. These include the sustainable production of biofeedstocks and their conversion into bioproducts, biomaterials, biofuels and bioenergy. These complement more applied missions by DOE's Office of Energy Efficiency and Renewable Energy. Neutron methods have applications in non-destructively probing the structures of the biofeedstocks (i.e. plant cell walls), their intermediates (e.g., pretreated biomass) and the final products (i.e., biomaterials) as well as in the biology that carries out this biosynthesis and bioconversion. The structural biology aspect crosses scales to include key enzymes, complexes, transporters, membranes, polymers and fibers.

Brief summary of the plenary talks

Both of our plenary speakers have been collaborators and users of neutron beamlines at ORNL user facilities. Both provided quad charts which are in the supplemental material.
Dr. Shishir Chundawat, Rutgers University, spoke on "Using neutrons to visualize biomass biosynthesis and deconstruction by biological systems." He gave examples in glycan biosynthesis and conversion from plant cell walls. Specifically looking at the polymer structures with carbohydrates and lignin under various pretreatments. He brought up a new related area to plastics upcycling and reuse by biology. At the enzyme level he gave examples of enzyme supercharging as well as multimodal visualization of cellulase activity on cellulose surfaces. This approach could also be applied to plastic degrading enzymes on plastic surfaces in the future.

Dr. Breeanna Urbanowicz, University of Georgia-Athens, spoke on "Valorization of plant glycopolymers for biomaterial applications". A key statement in her talk was that "Unlike DNA, RNA, and proteins, complex carbohydrates are not defined by sequence-based templates but are synthesized through the concerted actions of enzymes that are able to achieve synthesis of precisely structured polymers." She gave examples of the structure of complex carbohydrates with specific examples of how side-chains influence structure and conversion. These modifications alter biopolymer-biopolymer interactions with macroscale results in both structure, such as fibril organization, and reactivity, such as convertibility of monomers or directly into biomaterials. The structures and specificity of biosynthetic enzymes are also of critical interest. The potential to visualize in situ or in vivo reactions and changes in structures using the nondestructive and penetrating nature of neutrons was highlighted.

Discussions on charge questions

The breakout session started with a discussion on research needs and emerging science areas. A better understanding of molecular mechanisms of depolymerization by cellulolytic enzymes, which are relevant in the conversion of lignocellulose to value-added products, is needed. There is particular interest in molecular mechanisms governing differences in processing of amorphous and crystalline regions and aggregation processes during the conversion of lignocellulosic materials. These research needs also apply to interfacial active enzymes relevant to plastics upcycling. Biological processes and mechanisms involved in bioplastics deconstruction and utilization are emerging research topics. Working with such highly complex and dynamic biological systems requires the use of multimodal techniques that integrate neutron scattering, neutron diffraction with laboratory techniques such as spectroscopic techniques and other analyses.

Solvents are a key component of many chemical processes and a detailed understanding of the role of solvent dynamics on both biomaterials and enzymes is needed. Water and solvent dynamics govern structure-function relationships, which drives a need for a deeper understanding of how solvent interactions at solid-liquid interfaces affect catalytic reactions.

Biopolymer formation in plants is facilitated by enzyme complexes that synthesize biopolymers and assemble them in defined ultrastructures. However, the underlying molecular mechanisms are not fully understood as to how these large multi-domain enzyme complexes synthesize polysaccharides and biopolymers. Visualization of protein complexes in vivo, in membranes or interacting with solid substrates could provide mechanistic insights into these critical processes, such as the dynamics of intrinsically disordered proteins or polymer-polymer interactions during synthesis and assembly into defined structures. In addition, the preparation of functional and biocompatible polymer materials from biomacromolecules is gaining significant interest in recent years. Probing the structure of these novel polymers, such as derivatives of xylan-rich hemicelluloses is of particular interest and could be accomplished through the study of colloidal particle structures, e.g. xylan-methacrylate or xylan-urethane polymers, in different solvents. Understanding the structure-property relations of functionalized biopolymers, whether they were obtained by chemical or enzymatic modifications could result in significant advances.

Overall, new tools are needed that reveal static and dynamic structures of composites and laminates in biomass, plastics, and biomaterials. There was a consensus that studying the dynamics of these structures across broader time and length scales (i.e., a growing plant) is highly desirable. The greater neutron flux provided by STS is expected to lead to substantial improvements with respect to spatial and temporal resolution.

Deuterium labeling takes advantage of the particular sensitivity of neutrons to the isotopes of hydrogen and thus are an essential tool for the study of complex assemblies, structures and dynamics. The participants discussed potential use cases for deuterium labeling and identified potential research and development needs. Use cases include the selective characterization of a specific polymer within a mixture of components such as constructed or synthesized composites. Moreover, in-vivo labeling of cells using selective tags for visualization of molecular components or substrates in cell walls, membranes, single cells or biofilms could facilitate their characterization. For example, tags that can be linked using click chemistry or incorporated with genetic engineering could be used to target specific processes. Some of these advanced labeling strategies require the availability of highly specialized deuterated substrates. Although many deuterated chemicals are available from commercial sources, their use can be prohibitively expensive for many types of experiments.

Advances in sample preparation and novel sample environment capabilities are required to enable complex experimental designs. The participants discussed the use of STS beamlines offering high brightness in conjunction with pump-probe approaches to observe dynamic protein configuration changes at timescales of seconds or faster. These advances could enable the observation of complex biological processes in vivo, such as protoplast cultivation and differentiation in plants. The participants also discussed specific sample environment needs for ex situ and in situ experiments at STS. Sample environments that allow accurate control of environmental parameters, such as anaerobic conditions, gas phase composition (e.g. CO_2, O_2), and temperature are desirable. The group also discussed sample environments for in vitro studies and included capabilities for sample additions during experiments such enzyme reaction cascades, platforms for observing protein complexation and equipment for supporting studies using templating and polymer patterning. For in vivo studies, the desire longer time course experiments and the accommodation of larger samples sizes was expressed. These capabilities would enable intermittent "daily" measurement of growth in whole plants and studies of the dynamics during photosynthesis of plant cells or of photosystems (separate or in membrane).

The discussion on science-driven capabilities focused on multimodal techniques complemented by offline analysis capabilities. This need arises from the fact that neutrons probe the entire samples, but multimodal techniques are needed to validate features of interest complementing structure and dynamics information obtained from scattering. Fourier-transform infrared (FTIR) spectroscopy and fluorescence spectroscopy were identified as priorities as these techniques offer valuable complementary insights into the chemistry and electronic structure of the sample. More specific examples include equipment and supplies to work with membranes, micelle systems and scaffolds. The availability of offline and real-time modeling capabilities links, such as molecular dynamics simulations and modeling of neutron scattering data would enable testing and validation of various experimental scenarios (e.g. sample states and conditions, signal-to-noise ratio and identification of initial parameters for fitting neutron scattering data).

In addition, facile access to offline analysis capabilities, such as cryo-EM, SIMS, Raman spectroscopy, mass spectrometry, and computational models (MD, QM/MM) are desirable for targeted sample characterization that complement neutron scattering, diffraction, or imaging data. Multimodal capabilities are expected to increase productivity as multiple data sets can be collected at once on the same sample. Moreover, such techniques enable continuous monitoring, steering and validation of sample chemistry during the experiment and allow immediate detection of conditions that may have adverse effects on the results of the neutron scattering experiment.

Finally, the discussion focused on how STS could enable exploratory experiments and proof-of-concept work. The proposed approach includes an initial consultation, a quick "turn-around" through mail-in system for preliminary exploratory experiments. Moreover, increased attention should be directed towards "novice users" to help them identify the best conditions and beam line based on a series of preliminary data obtained using widespread techniques to frame optimal parameters for neutron scattering or imaging. For example, preliminary SAXS data can be collected to project and properly design SANS experiments.

4.6 Topic 6: Geochemistry: Nanoscale structure of porous materials

Brief summary of the plenary talks

The strong interaction between neutron and a material's nuclei makes neutron a unique probe to characterize a material's structural properties and dynamic processes. The applications in earth materials ranges from mineralogy (structure determinations of single crystals to short-range order in amorphous materials), petrology (mineral physics and magnetic structure under high pressure/temperature), rheology (anisotropy, strain and stress), and geochemistry (fluid-rock interaction). Despite the unique opportunities provided by neutron facilities, the earth science users are still a small minority (e.g. the number of submitted proposals in earth science for neutron scattering beamlines). The focus of the workshop was the opportunities and barriers of neutron facilities to earth scientists.

The first speaker for this session was Prof. David Cole, who is an Ohio Research Scholar at Ohio State University. Since the 1990s, his group has been the leading group to use neutron scattering tools to study complex fluid-gas-matrix interactions. His presentation focused on the use of elastic, quasi-elastic and inelastic neutron scattering to understand the structure and dynamics of systems that are important to geochemists, including ion association in complex aqueous solutions, solvent-exchange reactions at mineral–water interfaces, and reaction and transport of fluids in nanoporous materials. He gave specific examples about the behaviors of hydrocarbon molecules confined in silica nanopores (e.g., hydration, diffusion) by quasi-elastic neutron scattering.

The second speaker for this session was Dr. Dan Hussey, who is team leader at NIST Center for Neutron Research and an expert in neutron radiography and tomography in different materials including geomaterials. Dan's talk focused on the physical basis of neutron microscopy and wavelength-selective neutron imaging (Bragg-edge and dark field imaging) for multiscale images. He gave application examples such as transport and reaction of gas in cement and water infiltration in concrete.

Discussions on charge questions

Following the plenary talks, the participants met to discuss several topics. Most participants agreed that the characterization of pore structures of rocks by small-angle neutron scattering, and ultra-small-angle neutron scattering has been boosted, partially driven by the need to better understand the pore structure of shale, formations related to unconventional gas and oil development. On the other hand, much quasi-elastic and inelastic neutron scattering work needs to be done to understand the dynamics and reactions occurring at fluid-solid interfaces. Some interesting topics include: 1) Track interfacial reactions in real time to separate the roles of kinetic versus thermodynamic behavior; 2) Constrain elementary mechanisms of how fluid-solid interface responds near or far from equilibrium; 3) Translate molecular-scale description of interfaces to thermodynamic or kinetic quantities for the purpose of linking to meso- to macroscopic models (how to across multi-domains). Another interesting topic is fluid and mineral behavior at extreme conditions, which is still a wide-open area of fundamental research in earth science. Example questions related to phase transition in extreme conditions: what triggers the phase change? What is the role of defects or hydrogen? How are chemical and mechanical processes coupled during a phase transition? Due to the high sensitivity to hydrogen, neutron scattering provides a unique probe to detect hydrogen location, its bonding configuration, and the dynamics in minerals. This information is critical to design geological hydrogen storage.

The group then discussed the essential requirements and capabilities that should be incorporated into the neutron beamlines. Multi-scale characterization is important, such as Broadband Wide-Angle VElocity Selector spectrometer (BWAVES, time scale) and small- and wide-angle neutron scattering diffractometer/spectrometer (CENTAUR, length scale). Crystal phase identification from Bragg edge imaging and diffraction from multi-phase minerals is still challenging. Accessing QENS and reflectometry beamtime seems still not easy for the geochemistry community. A special cell that allows in-situ characterization at extreme conditions is desirable.

APPENDIX A. WORKSHOP AGENDA

Second Target Station/Biological and Environmental Systems Science Workshop

June 14, 21, and 28, 2022

1-5 pm EDT, Virtual Meeting

Time (EDT)	Event/Activity	Lead
Day 1, June 14 (1-5pm EDT) **Meeting Link: Click HERE** **Topic 1: Soil, rhizosphere processes and plant-soil interactions – Click HERE** **Topic 2: Biological systems, synthetic biology and enzyme engineering – Click HERE**		
1:00pm – 1:20pm	Welcome & Introduction to STS	Eric Pierce, Ken Herwig, Shuo Qian, Alex Johs
1:20pm – 1:40pm	Plenary Talk 1 Christian Toetzke, University of Potsdam	Jeffrey Warren
1:40pm – 2:00pm	Plenary Talk 2 Mutez Ali Ahmed, University of California, Davis	Jeffrey Warren
2:00pm – 2:20pm	Plenary Talk 3 Jonathan Nickels, Univ. of Cincinnati	James Elkins
2:20pm – 2:40pm	Plenary Talk 4 Yannick Bomble, NREL	James Elkins
2:40pm – 2:50pm	Break for 10 minutes	
2:50pm – 4:50pm	Concurrent Session 1: Soil, rhizosphere processes & root water dynamics: plant-soil interactions, water uptake and transport	Jeffrey Warren
2:50pm – 4:50pm	Concurrent Session 2: Biological systems, synthetic biology and enzyme engineering	James Elkins
4:50pm – 5:00pm	Session chair summary	
5:00pm	Adjourn	

Time (EDT)	Event/Activity	Lead
Day 2, June 21 (1-5pm EDT) **Meeting Link: Click HERE** **Topic 3: Plant-microbe interactions – Click HERE** **Topic 4: Biopreparedness – Click HERE**		
1:00pm – 1:20pm	Welcome, Quick recap on Charges	Eric Pierce, Ken Herwig, Shuo Qian, Alex Johs
1:20pm – 1:40pm	Plenary Talk 1 Michelle Buchanan, ORNL	Leighton Coates
1:40pm – 2:00pm	Plenary Talk 2 Kenneth Kemner, ANL	Jennifer Morrell-Falvey
2:00pm – 2:20pm	Plenary Talk 3 Marcus Cicerone, Georgia Institute of Technology	Jennifer Morrell-Falvey
2:20pm – 2:40pm	Plenary Talk 4 Susan Tsutakawa, LBNL	Leighton Coates
2:40pm – 2:50pm	Break for 10 minutes	
2:50pm – 4:50pm	Concurrent Session 3: Plant-microbe interactions	Jennifer Morrell-Falvey
2:50pm – 4:50pm	Concurrent Session 4: Biopreparedness	Leighton Coates
4:50pm – 5:00pm	Session chair summary	
5:00pm	Adjourn	

Time (EDT)	Event/Activity	Lead
Day 3, June 28 (1-5pm EDT) **Meeting Link: Click HERE** **Topic 5: Next generation bioproducts, biomaterials and bioenergy – Click HERE** **Topic 6: Geochemistry – Click HERE**		
1:00pm – 1:20pm	Welcome, Quick recap on Charges	Eric Pierce, Ken Herwig, Shuo Qian, Alex Johs
1:20pm – 1:40pm	Plenary Talk 1 Shishir Chundawat, Rutgers	Brian Davison
1:40pm – 2:00pm	Plenary Talk 2 Breeanna Urbanowicz, Univ. of Georgia	Brian Davison
2:00pm – 2:20pm	Plenary Talk 3 Dave Cole, OSU	Xin Gu
2:20pm – 2:40pm	Plenary Talk 4 Dan Hussey, NIST	Xin Gu
2:40pm – 2:50pm	Break for 10 minutes	
2:50pm – 4:50pm	Concurrent Session 5: Next generation bioproducts, biomaterials and bioenergy	Brian Davison
2:50pm – 4:50pm	Concurrent Session 6: Geochemistry	Xin Gu
4:50pm – 5:00pm	Session chair summary	
5:00pm	Adjourn	

APPENDIX B. WORKSHOP PARTICIPANTS

Session moderators

Topic	Session	Organizer	Affiliation
1	Soil, rhizosphere processes and plant-soil interactions	Jeff Warren	ORNL
2	Biological systems, synthetic biology and enzyme engineering	James Elkins	ORNL
3	Plant-microbe interactions	Jennifer Morrell-Falvey	ORNL
4	Biopreparedness	Leighton Coates	ORNL
5	Next generation bioproducts, biomaterials and bioenergy	Brian H. Davison	ORNL
6	Geochemistry	Xin Gu	ORNL

List of registered workshop participants

Name	Affiliation
Alan Hicks	ORNL
Alexander Johs	ORNL
Andrey Kovalevsky	ORNL
Breeanna Urbanowicz	University of Georgia
Brian Davison	ORNL
Brian Sanders	ORNL
Changwoo Do	ORNL
Christian Tötzke	University of Potsdam
Christina Hoffmann	ORNL
Christoph Wildgruber	ORNL
Daniel Hussey	NIST
David Cole	Ohio State
David Graham	ORNL
Dima Bolmatov	ORNL/UTK
Elizabeth Herndon	ORNL
Eric Pierce	ORNL
Eugene Mamontov	ORNL
Gergely Nagy	ORNL
Hassina Bilheux	ORNL
Honghai Zhang	ORNL

Hugh O'Neill	ORNL
James Elkins	ORNL
Jason Gardner	ORNL
Jeff Warren	ORNL
Jennifer Morrell-Falvey	ORNL
Jeremy Smith	ORNL/UTK
Jiafu Mao	ORNL
Jiao Lin	ORNL
John Ankner	ORNL
Jonathan Nickels	Univ. of Cincinnati
Joseph Pu	ORNL
Ken Kemner	Argonne National Laboratory
Kenneth Herwig	ORNL
Laura-Roxana Stingaciu	ORNL
Leighton Coates	ORNL
Manjula Senanayake Mudiyanselage	ORNL
Marcus Cicerone	Georgia Institute of Technology
Marissa Mills	ORNL
Michelle Buchanan	ORNL
Mitch Doktycz	ORNL
Mutez Ali Ahmed	University of California, Davis
Natalie Griffiths	ORNL
Rui Zhang	ORNL
Salik Dahal	ORNL
Serena Chen	ORNL
Shishir Chundawat	Rutgers University
Shuo Qian	ORNL
Susan Tsutakawa	Lawrence Berkeley National Laboratory
Udaya Kalluri	ORNL
Xianhui Zhao	ORNL
Xiao-Ying Yu	ORNL
Xin Gu	ORNL
Yannick Bomble	National Renewable Energy Laboratory
Yaohua Liu	ORNL
Yuxuan Zhang	ORNL

APPENDIX C. QUAD CHARTS PROVIDED BY PARTICIPANTS

Quad Chart: Valorization of plant glycopolymers (Breeanna Urbanowicz)

Opportunity

- Understanding the relationship between polysaccharide structure (DP, substituent amount and identity) and functionalized material properties.
- Study Xyla-methacrylate or Xyla-urethane colloidal particle structures in different solvents (core-shell).
- Increased understanding of polymer-polymer interactions during synthesis and assembly.
- Probe biological processes of bioplastic deconstruction and or utilization. Can we probe complex and dynamic living systems?

Importance of neutrons

- Contrast: selectively deuterate polysaccharides or support structures/scaffolds (i.e. cellophane, PLA)?
- Non-destructive, samples can be recovered.
- Electrically neutral, facilitating penetration and analysis across a broad range of length scales.
- Fast time frames!

Neutron scattering tools needed

- Tools to study polymer synthesis, polymer deconstruction, and polymer-polymer interactions in real time, during in vitro/ ex situ synthesis and breakdown as interactions occur.
- Small samples for multimodal analyses, molecular-level dynamics of microbes (BWAVES)
- Time resolved measurements: kinetics (CENTAUR), crystallization (PIONEER), diffusion (QIKR)
- Water, material components, porosity, and carbon during complex biological deconstruction (CUPI^{2}D)

Broader sciencific impact

- Understanding material behaviors at fundamental levels will enable design of improved polysaccharide valorization strategies.
- Gaining enhanced insights into structure-property relationships of chemically functionalized and/or enzymatically synthesized polymers.
- Understand bio-deconstruction vs bio-utilization.
- Design materials for targeted end of life uses.

Quad Chart: Visualizing cellulolytic enzyme activity using multimodal techniques (Shishir Chundawat)

Opportunity

- Understand molecular mechanisms of cellulolytic enzymes relevant to lignocellulose upgrading to value added products
- Understand molecular mechanisms of other interfacial active enzymes relevant to plastics upcycling

Importance of neutrons

- Penetrate optically opaque samples like biomass and plastics
- Use deuterated proteins and/or substrates to achieve contrast
- Increased S/N over current neutron imaging methods

Neutron scattering tools needed

- SANS/WANS
- High spatiotemporal resolution needed to image enzyme-cellulose binding interactions

- Potential integration of advanced microscopy (e.g., fluorescence, tweezers) with neutron scattering to enable multimodal imaging

Broader scientific impact

- Understand how enzymes behave at solid-liquid interfaces to achieve catalytic turn-over
- Engineer more efficient enzymes for lignocellulosic and plastic based waste materials upcycling to value added products (e.g., biofuels)

Quad Chart: Visualizing biomass and plastics dissolution in solvents (Shishir Chundawat)

Opportunity

- Understand molecular mechanisms of how solvents and chemical processes can be developed for lignocellulose & plastics upgrading to value added products

Importance of neutrons

- Penetrate optically opaque samples like biomass and plastics
- Useful when X-rays cannot be used with scattering solvents
- Use deuterated solvents and/or substrates to achieve contrast
- Increased S/N over current neutron imaging methods

Neutron scattering tools needed

- SANS/WANS for kinetic studies
- Need for integration of in-situ pretreatment capabilities
- Improved temporal resolution
- Ability to introduce solvent/samples into chamber during imaging
- Need precise control over humidity

Broader scientific impact

- Understand polymer dynamics in novel solvent systems for biomass or plastics dissolution
- Engineer more chemical processes for lignocellulosic and plastic based waste materials upcycling to value added products (e.g. biofuels)

Quad Chart: Visualizing cell wall and biopolymers synthesis in real-time (Shishir Chundawat)

Opportunity

- Understand molecular mechanisms of how plants (and other cellular systems in general) synthesize biopolymers and assemble them in defined ultrastructures
- Understand how large multi-domain (and often membrane embedded) enzymes synthesize polysaccharides

Importance of neutrons

- Penetrate optically opaque samples like single cells
- Useful when X-rays cannot be used with scattering solvents
- Use deuterated solvents and/or substrates to achieve contrast
- Increased S/N over current neutron imaging methods
- Neutrons are less damaging to living systems compared to X-rays

Neutron scattering tools needed

- SANS/WANS for kinetic studies
- Potential integration of in-situ imaging capabilities with other imaging modalities (e.g., optical)
- Ability to keep plant cells healthy and capable of growth in imaging chamber
- Need higher resolution for single cells

Broader scientific impact

- Understand enzyme action at single cell level for polymer synthesis
- Understand biopolymer dynamics during self-assembly in vivo

- Engineer more efficient plants and other enzymatic systems capable of synthesizing polysaccharides & biopolymers more efficient

ACKNOWLEDGEMENTS

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