3.5 Biological Systems: Revolutionizing Health and Biomaterials

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Introduction

The transformative potential of neutron science in advancing biology is both immense and urgent. Neutrons possess unique properties that are crucial for unraveling the multiscale phenomena underlying biological processes across diverse time and length scales. These properties are harnessed through a broad range of techniques that serve diverse biological research communities, offering unparalleled insights into molecular structures and dynamics.

Among these techniques, neutron crystallography stands out as the only method capable of precisely locating every proton within macromolecules. This capability is critical for understanding biological mechanisms, as hydrogen atoms—comprising nearly half of the atoms in proteins—are key players in mediating hydrogen bonds, nonbonding interactions, and three-dimensional structures essential for function. Their roles are fundamental to the energy landscapes and mechanisms driving substrate binding, proton transfer, and enzymatic catalysis.

Small-angle neutron scattering (SANS) complements this by providing unique structural insights into complex biological assemblies and solution structures. Leveraging isotopic labeling and the differential sensitivity of neutrons to hydrogen and deuterium, SANS reveals information about biomolecular arrangements that other techniques cannot achieve. Furthermore, the energy scales of neutrons are matched to the motions of atoms and molecules, enabling the study of hydrogen dynamics and structural motions in biomolecules and assemblies with unmatched precision. This sensitivity to hydrogen and deuterium allows for isotopically resolved studies of processes such as reaction kinetics, membrane behavior, and dynamic phenomena, offering a real-time view of critical biological mechanisms.

Neutron-based techniques, therefore, occupy a unique position in biology, offering unparalleled capabilities to resolve hydrogen's role in molecular interactions and mechanisms. Yet, the full potential of these approaches has been constrained by limitations in neutron sources and instrumentation.

The Second Target Station (STS) at the Spallation Neutron Source (SNS) is poised to overcome these barriers. By addressing limitations in signal-to-noise ratios, data acquisition times, and intensity at longer neutron wavelengths, the STS will revolutionize neutron science. These advancements will significantly expand the reach of neutron techniques, fostering breakthroughs in biology and attracting a new generation of interdisciplinary scientists to tackle the grand challenges of biological systems. Over the next decade, the STS promises to drive a transformative shift in our ability to probe the molecular foundations of life.

Grand Challenges

Engineering the Principles of Life (Synthetic Biology)

Understanding how hydrogen bonds contribute to the specificity, stability, and kinetics of enzymatic reactions, deciphering how protons move through complex protein systems, the role of structured water in stabilizing protein complexes, ribosomes, or viral capsids, subtle hydrogen bond rearrangements in allosteric or conformational changes, and understanding hydrogen dynamics in natural biological systems, are all key to understanding mechanism and using that understanding to enabling engineering biology for technological applications. Advanced neutron imaging techniques can visualize the distribution and dynamics of hydrogen in cells and organelles, providing unprecedented insights into processes such as cell division, organelle biogenesis, and intracellular transport. Understanding how lipids modulate protein function in membranes is critical for comprehending processes like signal transduction and vesicle fusion. Neutron reflectometry and scattering can reveal the role of hydrogen in lipid-protein interactions and membrane organization. Neutron studies through multiple techniques enable us to uniquely probe these questions and address this challenge.

Biosecurity and Biopreparedness

Biosecurity -protecting against biological threats - has never been more critical. Neutron scattering has already played a role in developing lipid nanoparticle (LNP) delivery systems for mRNA vaccines, a game-changer in the fight against infectious diseases like COVID-19. Neutrons can reveal detailed structural and functional information about hydrogen-rich biothreat agents, such as toxins or pathogenic proteins, essential for designing neutralizing agents and protective strategies. The can be used to optimize hydrogen bonding and hydration properties in proteins used for vaccines and monoclonal antibodies, ensuring they remain stable during storage and distribution. Hydrogen dynamics play a key role in how pathogens survive under environmental stress. Neutron techniques can uncover these mechanisms, aiding strategies to mitigate the spread of pathogens in water, soil, and air. Neutrons can probe hydrogen bonding in enzymes and microbial systems used for bioremediation, improving their ability to detoxify biothreat agents or environmental contaminants from biological attacks. Neutron techniques can elucidate how hydrogen dynamics in bacterial enzymes and membranes contribute to resistance mechanisms. This knowledge supports the development of new antibiotics or resistance-modulating therapies. Understanding hydration and hydrogen networks in stress-resilient microbes and biomolecules can inform the design of biological systems capable of functioning in extreme or contaminated environments, aiding bioremediation and biodefense.

Bioenergy and the Circular Bioeconomy

Enzymes play key roles in bioenergy production, such as breaking down lignocellulosic biomass for biofuel generation. Neutron studies can uncover how hydrogen bonds influence enzyme stability and efficiency, enabling the design of more robust biocatalysts for renewable energy applications. Cellular bioenergetics, such as proton-coupled electron transport in photosynthesis and respiration, are central to renewable energy strategies. Neutron scattering provides insights into hydrogen and proton dynamics in key systems like ATP synthase, facilitating advancements in bioinspired energy technologies. Hydrogen dynamics are critical for understanding catalytic processes in carbon capture, utilization, and storage (CCUS). Neutrons can study hydrogen positions and pathways in metalloenzymes and synthetic catalysts, advancing carbon-neutral energy solutions. Structured water and hydrogen bonds are vital for protein complex stability, influencing processes like carbon fixation and biopolymer assembly. Neutrons provide molecular-level insights into hydration dynamics, aiding in the development of bioinspired materials and systems environmental and energy needs. Photosynthesis underpins renewable energy strategies by informing solar fuel generation. Neutrons can reveal hydrogen dynamics in photosystem II and the oxygen-evolving complex, improving artificial photosynthetic systems and energy conversion efficiency. Whole-cell and organelle imaging with neutrons can visualize hydrogen-rich biomolecules, advancing systems biology models for microbial communities used in bioenergy production and waste bioremediation.

Understanding the Molecular Basis of Health, Aging, and Well-Being

Neutron techniques leverage isotopic substitution (e.g., deuterium labeling) to distinguish specific atomic interactions and hydrogen dynamics, enabling precise studies of metabolic pathways, signaling networks, and stress responses. Diseases such as Alzheimer’s and Parkinson’s involve protein misfolding and aggregation. Neutron scattering techniques can investigate hydrogen and hydration dynamics in amyloid fibrils or prion proteins, offering insights into the mechanisms underlying these pathologies. Many therapeutic drugs depend on hydrogen bonding with their targets. Neutron studies enable accurate characterization of these interactions, providing critical data to optimize drug binding, efficacy, and selectivity, particularly for challenging targets like G-protein-coupled receptors (GPCRs). A deep understanding of the relationship between genotype and phenotype - health vs. disease - will transform personalized medicine. While X-ray techniques have solved nearly 190,000 structures, they lack information on hydrogen atoms, which are essential for understanding ligand binding, protein interactions, and enzyme mechanisms.

Enhancing Accessibility for the Expanding Scientific Community

With higher brightness, experiments that currently require days or weeks to collect sufficient data can be completed in hours or even minutes. This drastically reduces beamline time requirements, allowing more researchers to access neutron facilities. The increased flux allows for the analysis of smaller or lower-concentration biological samples, which are often challenging to study with current facilities. This is critical for investigating rare, precious, or difficult-to-crystallize biomolecules. Higher brightness enables the use of advanced techniques such as neutron spin echo, neutron reflectometry, and small-angle neutron scattering (SANS) with unprecedented resolution and speed. This opens up new possibilities for studying complex biological phenomena. Brightness improvements make it feasible to study biological systems under more native-like conditions, such as whole cells or organelles, rather than relying solely on isolated components. This bridges the gap between in vitro and in vivo studies. The higher intensity of neutrons makes it possible to conduct high-resolution time-resolved studies of fast biological processes, such as enzyme-substrate interactions, proton transfer, or allosteric signaling. The Increased brightness enables high-throughput workflows, such as screening multiple conditions for drug binding, enzyme activity, or protein-ligand interactions. Faster experiments and reduced sample size requirements lower the barriers for smaller research groups, early-career scientists, and underrepresented institutions to access neutron facilities. Shorter experiment times and higher success rates make neutron facilities more accommodating for training students and postdocs, creating a pipeline of skilled researchers. This democratizes neutron science.

Instrumentation requirements to address grand challenges

Neutron crystallography. The number of structural models produced from neutron diffraction studies has been steadily increasing with on average, 15 new models per year over the past decade, 30% of all those from the SNS. The current limitations are the volume of the sample required and the exposure time needed for a dataset. While this has not limited key studies on systems like superoxide dismutase, polysaccharide monooxygenase, maltotetraose, and critical efforts to fight COVID amongst others, they significantly limit the ability to make anl impact in the manner that X-ray structures have been harnessed by AI, reflected in the 2024 Nobel Prize in Chemistry. An analysis of the MASSIF synchrotron X-ray beamline at ESRF shows that out of ~44,000 crystals characterized in both volume and resolution, only about 2,000 of them, 5%, would be regarded as suitable of analysis at the SNS generously based on the assumption of a 0.008 mm^3 volume, and full per-dueteration of the protein. If the brightness could be raised even 50-fold with the STS, that number would rise to almost 10,000 crystals suitable for study, 23% of the crystals typically seen by a synchrotron beamline. This assumes the current exposure times, if larger crystals were used, the data collection time would be reduced and the 15 new models a year with 30% coming from the SNS could be raised at least 5-fold, with the SNS contributing at least 70% of the world's new neutron structural data from the EWALD line alone. A factor of 100 increase in brightness considerable improves this making the

SNS with the STS the world leader in this area, even with other planned facilities on the horizon. It would give the STS the ability to address systems that cover multiple grand challenges in a single year.

Small and Wide-Angle Neutron Scattering

The creation of the high-brightness cold neutrons in the STS will be leveraged to create high resolution SANS instrumentation with world leading data collection rates. The use of cold neutrons for time-of-flight SANS experiments allows for excellent energy and spatial resolution. Cold neutrons are uniquely important for maximizing Time-of-Flight SANS instruments. Cold neutron will improve the way data is processed and recombined, will enable larger structures and assemblies to be measured regularly, and will allow for novel data treatments dealing with inelastically scattered neutron – a significant background issue in many experiments. Indeed, the later point is a potentially unique advantage which can be realized for Time-of-Flight SANS instruments. It may also be desirable to optimize for improved flux on smaller samples to use less material – a frequent bottleneck for the biological community. Anticipated improvements in instrument design are also exciting and promise to multiply those realized from the new source itself. When combined, there will be material improvements in both the quantity and quality of experiments performed with this technique.

Neutron Imaging

The realization of high-flux cold neutron sources (the STS) aligns favorably with recent developments in neutron focusing optics. Potentially revolutionary improvements over pinhole-camera designs of current neutron imaging may be made using focusing optics based on Wolter optics. The spatial resolution of neutron images has progressed over time, but the goal of a neutron microscope with micron scale spatial resolution is still out of reach. The combination of these advances in instrumentation with high intensity cold neutrons is needed to realize the goal of a neutron microscope for biological and other materials.

Inelastic Scattering

The suite of inelastic neutron scattering instruments enabled at a new high intensity cold neutron source will simply be world class. The nature of the source is ideal for inelastic scattering and will improve the temporal resolution and data acquisition rates for all inelastic scattering experiments. This will enable the user community to measure nanosecond scale motions in a range of biological materials. Proposed inelastic instruments such as BWAVES, CHESS, and EXPANSE are well suited to cover biological motions from picosecond scale molecular vibrations to near-nanosecond scale diffusional motions of solvents and metabolites, to the many-nanosecond scale domain motion of proteins, nucleic acid chains, and lipid membranes. As with discussions of other scattering techniques, there will be circumstances where improvements to flux will be prioritized – allowing smaller samples for precious and rare biological samples to be observed. This is a critical improvement due to the advantages and specificity of isotopically labeled materials. The combination of a strong biodeuteration program with higher flux, higher resolution neutron spectroscopy will tell us more about the way lipids, proteins, nucleic acids, metabolites and solvents move at the molecular time and length scale. Truly, the next dimension in structural biology.

Neutron Polarization

Neutron polarization ratios using common ^{3}He spin flippers are strongly wavelength dependent, with long-wavelength, or cold, neutrons providing greater polarization. Spin filters based on this principle can cover a large solid angle and are ideal for use in time-of-flight instruments. Polarization of incident and scattered neutrons comes at a great cost in terms of flux. Improvements in both flux, and polarization ratios due to the nature of the new source will enable completely new data analysis method and make new classes of experiments possible.

Summary

The upcoming Second Target Station (STS) holds the promise of significant impact on biological science. Neutron crystallography, currently constrained by large sample volumes and long exposure times, would see a dramatic expansion in applicability. A 100x brightness increase would enable the study of significantly smaller or lower-concentration samples, increasing the proportion of viable crystals for analysis from 5% to over 25% of those able to be characterized at leading synchrotron beamlines. This could elevate the annual output of new structural models by at least 5-fold, placing SNS as a global leader. The enhanced ability to precisely locate hydrogen atoms, critical for understanding hydrogen bonds, proton transfer, and enzymatic mechanisms, would provide insights into processes central to energy landscapes, substrate binding, and catalysis, and provide critical knowledge to improve computational modeling approaches.

Higher neutron brightness enables time-resolved studies of fast biological processes, such as enzyme-substrate interactions and allosteric signaling, with unmatched resolution. Techniques like small-angle neutron scattering (SANS) and neutron spin echo would benefit from increased flux, offering detailed views of hydrogen dynamics and biomolecular interactions in near-native environments. Whole-cell and organelle imaging would become feasible, bridging the gap between isolated and in vivo studies.

Neutrons enable the understanding of hydrogen dynamics in pathogens, aiding vaccine and monoclonal antibody design while supporting bioremediation and biodefense strategies. Insights into hydrogen bonding and dynamics in enzymes, photosynthesis, and carbon capture processes would drive innovations in renewable energy and bioinspired systems. Neutron studies could elucidate mechanisms behind diseases like Alzheimer's and Parkinson's by investigating protein misfolding and aggregation, while optimizing drug binding for challenging targets. By integrating the benefits of enhanced brightness into diverse techniques such as neutron crystallography, SANS, and neutron imaging, the STS will redefine the scope of biological research, transforming our ability to understand and engineer life at the molecular level.