American Conference on Neutron Scattering

Biology and Biotechnology

* Invited Paper

SESSION D02.01: Biology and Biotechnology I

D02.01.01*
Studying Excipient Modulated Colloidal Stability and Viscosity of Monoclonal Antibody Formulations Using Small Angle X-Ray/Neutron Scattering
Amy Y. Xu[1,2], Maria M. Castellanos[3], Kevin Mattison[4], Susan Krueger[2] and Joseph Curtis[2]; [1]University of Maryland, United States; [2]National Institute of Standards and Technology, United States; [3]GlaxoSmithKline, United States; [4]Malvern Panalytical, United States

Excipients are substances that are added to therapeutic products to improve stability, bioavailability, and manufacturability. Undesirable protein-protein interactions (PPI) can lead to self-association and/or high solution viscosity in concentrated protein formulations that are typically greater than 50 mg/mL. Therefore, understanding the effects of excipients on non-specific PPI is important for more efficient formulation development. In this study, we used National Institute of Standards and Technology monoclonal antibody (NISTmAb) reference material as a model antibody protein to examine the physical stability and viscosity of concentrated formulations using a series of excipients, by varying pH, salt composition, and the presence of co-solutes including amino acids, sugars, and non-ionic surfactants. Small-angle X-ray/Neutron scattering (SAXS/SANS) together with dynamic/static light scattering (DLS/SLS) were used to obtain various experimental parameters to characterize excipient modulated PPI. In particular, the detailed analysis of effective structure factor $S(q)_{eff}$ measured from SAXS/SANS enabled the dissection of net PPI into hydrodynamic forces due to excluded volume as well as any additional attractive or repulsive interactions with the presence of excipients. The use of $S(q)_{eff}$, interaction parameter k_D, and second virial coefficient B_{22} as predictors for solution viscosity was also evaluated by comparing the predicted results with the measured viscosities in this study.

D02.01.02
The Nanostructure and Dynamics of Therapeutic Monoclonal Antibody Formulation in Solution Resolved by Neutron Spin Echo
Norman Wagner[1], Yun Liu[2,1] and Jannatun Nayem[1]; [1]University of Delaware, United States; [2]National Institute of Standards and Technology, United States

Monoclonal antibodies (mAbs) constitute a $105 Billion global market by providing unique therapeutic functionalities that hold great promises for personalized medicine. More than ten new mAb-based drugs were approved by FDA in 2017, bringing the total to 76, with these new drugs addressing diseases ranging from rheumatoid arthritis to high-risk neuroblastoma to multiple sclerosis. Manufacturing challenges and pharmacokinetics are often limiting the development of new mAb therapeutics. While the characterization of primary through quaternary structure is crucial for understanding their biochemical functionalities and efficacy, the quaternary structure and dynamics of therapeutic proteins in the solid state and in solution is crucial for understanding their stability in manufacturing, formulation, long-time storage, and during delivery. This work focuses on understanding the quaternary solution structure, protein-protein interactions (PPIs), and dynamics of the NIST standard reference mAb, NISTmAb RM 8671, in comparison to three industrial mAbs. The solution structure and interactions in buffer is characterized using small angle neutron scattering (SANS), while the dynamics in buffer are measured via neutron spin echo (NSE) and dynamic light scattering (DLS). The effects of concentration, temperatures, sugar, and salt conditions on the conformation, interaction, and

dynamics are explored for NISTmAb. The PPIs in solution exhibit strong temperature dependence for the concentrated samples driven by the competition of the charge repulsion and attraction. The diffusion interaction parameter, K_D, determined by DLS as a function of temperature further corroborates the temperature dependence of the PPIs observed by SANS. Measurements of the effective short time dynamics, D_{eff}, on timescales shorter than the structural relaxation but longer than the momentum and viscous relaxation time, as a function of concentration, temperatures, sugar, and salt content are performed by NSE. Analysis of the D_{eff} at the aforementioned conditions shows similar dependence across the reference and industrial mAbs. In addition to determining the relationship between translational dynamics and solution macroscopic behavior such as viscosity, another focus of this work is to understand the relation between the internal motions and the flexibility of mAbs as this is hypothesized to determine solution-state formulation stability. All atom MD simulations of NISTmAb is compared against NSE experiments. Analysis demonstrates the presence of strong internal motions that contribute significantly to the total effective diffusion coefficient. Further investigations are proposed to connect this motion to long-term mAb stability.

D02.01.03*
Isotopically-Labeled NISTmAb Enabled by Microbial Expression Host
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"NISTmAb", NIST Reference Material 8671, is a humanized IgG1κ monoclonal antibody (mAb) that is representative of current biologic pharmaceuticals and that has undergone extensive biophysical characterization using techniques including small-angle neutron scattering. However, work to-date has been limited to studies of the molecule at natural isotopic abundance. NISTmAb, like all currently FDA approved mAb biologics, was produced using a mammalian expression host. Mammalian hosts for mAbs offer high yields and biocompatibility for pharmaceutical use but are generally intractable for ^2H labeling due to the high cost of ^2H-labeled amino acids and the deleterious biological consequences of ^2H_2O for mammalian cell biology. Therefore, our laboratory has undertaken efforts to produce sequence-identical versions of the NISTmAb molecule in the ^2H-labeling amenable microbial hosts *Escherichia coli* and *Komagataella phaffii* (syn. *Pichia pastoris*). Efforts with *E. coli* have produced ^2H-labeled full-length "eNISTmAb" and antibody fragments in small yields and have highlighted known and previously-uncharacterized complications of mAb expression in bacteria. Current work with *K. phaffii* has focused on optimizing high-yield expression of the "yNISTmAb" antigen binding (fAb_1) fragment, prior to adaptation to ^2H_2O culture, through construct and host genetic manipulations supported by medium-throughput parallel screening. Future plans include the use of glycosylation pathway engineered *K. phaffii* hosts for full-length, N-glycosylated "yNISTmAb" expression.

D02.01.04*
An Ensemble of Flexible Conformations Underlies Mechanotransduction by the Cadherin–Catenin Adhesion Complex
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The cadherin–catenin adhesion complex is the central component of the cell–cell adhesion adherens junctions that transmit mechanical stress from cell to cell. We have determined the nanoscale structure of the adherens junction complex formed by the α-catenin●β-catenin●epithelial cadherin cytoplasmic domain (ABE) using negative stain electron microscopy, small-angle X-ray scattering, and selective deuteration/small-angle neutron scattering. The ABE complex is highly pliable and displays a wide spectrum of flexible structures that are facilitated by protein-domain motions in α- and β-catenin. Moreover, the 107-residue intrinsically disordered N-terminal segment of β-catenin forms a flexible "tongue" that is inserted into α-catenin and participates in the assembly of the ABE complex. The unanticipated ensemble of flexible conformations of the ABE complex suggests a dynamic mechanism for sensitivity and reversibility when transducing mechanical signals, in addition to the catch/slip bond behavior displayed by the ABE complex under mechanical tension. Our results provide mechanistic insight into the structural dynamics for the cadherin–catenin adhesion complex in mechanotransduction.

D02.01.05
AI-Driven Multi-Modal Workflow Incorporating Neutron Scattering to Resolve Intrinsically Disordered Protein (IDP) Structures
Debsindhu Bhowmik, Serena Chen and Christopher B. Stanley; Oak Ridge National Laboratory, United States

Biological macromolecules, namely proteins, RNA, DNA, lipids and carbohydrates, control how a living cell functions. These entities therefore are responsible for maintaining the health, growth and proper behavior of the cells in our body. Any imbalance in

their behavior or dysfunction would thus create serious health conditions including fatal diseases, like cancer, diabetes, Alzheimer's and other neurological disorders. Consequently, it is essential to map how the structural and dynamical properties of these bio-macromolecules influences the molecular mechanisms of their function. These are the questions that are yet to be fully answered, even with extraordinary progress in recent years in the experimental determination of protein structures and interactions using neutron/x-ray scattering techniques, NMR, cryo-electron microscopy, or by faster simulations with the help of more powerful computing resources and algorithms. The basic roadblocks have been (i) Modelling (connecting sequence to 3D structure), (ii) Efficiency (in handling large dataset with meaningful analysis), (iii) Scaling (integrate simulation to experiments in terms of time- and length-scales accessible to each of them) and (iv) State space exploration (map configuration space to identify intermediate states and generate novel ones by learning inherent distribution). In order to solve this problem, we are developing a completely unsupervised modular, multi-modal, high-throughput artificial intelligence (AI) driven framework that will integrate high volume experimental results to select the most appropriate modelling and simulation (M&S) method via active learning, and initiate further high performance computing (HPC) based deep learning (DL) techniques for resolving the bottleneck. Building this framework would serve in four ways - (a) In-depth knowledge, (b) Intelligent implementation, (c) Efficient computation and (d) Biological/medical application. As an example, with live demo, we will show how our general-purpose AI-driven multi-modal method, combined with state-of-the-art high-performance simulations, small-angle neutron scattering (SANS), and AI implementation uniquely resolves structures of intrinsically disordered protein (IDP) ensembles.

SESSION D04.01: Biology and Biotechnology II

D04.01.01*
A Mitochondrial Throttle—Lipid-Mediated Protein Complexes at the Mitochondrial Surface Studied by Neutron Reflectivity
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Mitochondria are not only "the powerhouses of the cell" but are also involved in multiple crucial cellular functions. Their dysfunction plays a central role in many disorders. Mitochondria organelles are composed of two membranes. The inner membrane plays a prominent role in power production via oxidative phosphorylation, while the mitochondrial outer membrane (MOM) acts as a "throttle", controlling the access of metabolites to the inner membrane and thus the rate of energy production. A significant portion of the control functions is carried on by the voltage-dependent anion channel (VDAC), a passive transport channel which allows water soluble metabolites and ions to cross MOM. Recent findings uncover an efficient regulatory mechanism of this channel through its interactions with cytosolic proteins. One such regulator is dimeric tubulin, which is best known as a fundamental unit of microtubules. A preponderance of evidence suggests that MOM binds dimeric tubulin, which blocks the flux of metabolites through VDAC by inserting its negatively charged, disordered C-terminal tail into the VDAC pore in response to a transmembrane potential. The discovery of this novel regulatory mechanism of mitochondrial respiration has raised several fundamental questions, including how tubulin binds to the MOM surface, whether its membrane-bound conformation is consistent with the accessibility of its C-termini to the membrane surface, and what role mitochondrial lipids assume in mediating the tubulin-VDAC interaction. In this talk, I will discuss of how we answer these questions by using a combination of electrophysiological, neutron reflectivity (NR), and molecular dynamics (MD) simulations studies. Probing the interactions of dimeric tubulin with VDAC by single-channel recordings we showed that tubulin induces transient blockages of the ionic current through the channel, identified as the insertion and escape of the unstructured charged C-terminal tail of tubulin into the channel. We determined the orientation of membrane-bound tubulin—and hence the face of the tubulin molecule that is in contact with the membrane surface—by NR on sparsely tethered bilayer lipid membranes. NR studies on tubulin-coated biomimetic membranes together with MD simulations reveal that dimeric tubulin is anchored to the membrane surface by an amphipathic α-helix of the α-tubulin subunit in an orientation that presents the C-termini of both tubulin subunits to the membrane surface. The structural model is supported by electrophysiological evidence using recombinant tubulin constructs, in which C-terminal tails bound to either subunit are individually observed in the VDAC nanopore. This study represents an essential step toward our understanding of the complex mechanisms of cytosolic peripheral membrane protein interaction with integral membrane proteins of mitochondria, which is important for the structure-

inspired design of mitochondria-targeting agents.

D04.01.02
Using Deuterium to Determine the Nanoscale Structure of Biomembranes
Jonathan Nickels[1], Frederick Heberle[2] and John Katsaras[3]; [1]University of Cincinnati, United States; [2]The University of Tennessee, Knoxville, United States; [3]Oak Ridge National Laboratory, United States

Biological membranes are complex, self-assembled systems of proteins, lipids, and carbohydrates, whose hierarchical organization is fundamental to physiological processes. In particular, the lateral organization of plasma membranes is now widely accepted as being an integral feature of biological function. Among the nanoscopic probes suitable for the study of biomembranes, neutrons stand out both for their non-destructive nature and their multi-scale spatial and temporal information content. Unlike x-rays, which interact with electrons, neutrons are scattered by atomic nuclei. Importantly, for the study of hydrogen-rich biomembranes, neutrons are not only sensitive to hydrogen, but can distinguish between the stable isotopes of hydrogen, namely protium and deuterium. In this talk, I will show how we used deuterium labeling and small angle neutron scattering to detect lipid domains in model [J. Am. Chem. Soc. 135, 6853 (2013)] and fully functional biomembranes [PLoS Biol. 15, e2002214 (2017)], and describe the need for chemical deuteration in order to make full use of the neutron's capabilities with regard to the study of complex biological systems and the development of techniques such as, neutron spin echo.

D04.01.03
Interfacial Interactions between Styrene-Maleic Acid Copolymers and Galactolipid-Containing Membranes—X-Ray and Neutron Reflectivity Studies
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Styrene-maleic acid (SMA) copolymers have recently gained attention for their ability to facilitate the detergent-free solubilization of membrane protein complexes and their native boundary lipids into polymer-encapsulated, nano-sized Lipid Particles, referred to as SMALPs. However, the interfacial interactions between SMA and lipids, which dictate the mechanism, efficiency, and selectivity of lipid and membrane protein extraction, are barely understood. Our recent finding has shown that SMA 1440, a chemical derivative of SMA family with a functionalized butoxyethanol group, was most active in galactolipid-rich membranes compared to other derivatives with the same styrene-to-maleic acid ratio (1.5:1). In present work, we have performed X-ray reflectometry (XRR) and neutron reflectometry (NR) on the lipid monolayers at the liquid-air interface following by the SMA copolymer adsorption. XRR and Langmuir $\Pi - A$ isotherms captured the fluidifying effect of galactolipids, which made SMA copolymers easily infiltrate into the lipid membranes. NR results revealed the detailed structural arrangement of SMA 1440 copolymers within the membranes and highlighted the role of butoxyethanol group in boosting the amphiphilicity of SMA 1440, which facilitates the copolymer insertion into the thylakoid lipid-containing membranes.

D04.01.04
SANS Probes of Nanoheterogeneities in Natural Plasma Membrane Models
Charles P. Collier[1], Dima Bolmatov[1], Maxim Lavrentovich[2], Michael Nguyen[3], Frederick Heberle[4], Christopher B. Stanley[1], Francisco Barrera[2], John Katsaras[1] and Drew Marquardt[3]; [1]Oak Ridge National Laboratory, United States; [2]The University of Tennessee, Knoxville, United States; [3]University of Windsor, Canada; [4]University of Tennessee, United States

Molecular heterogeneity and complexity at the nanoscale are defining characteristics of natural plasma membranes which biomimetic models have only partially emulated. Two of these, lipid transbilayer asymmetry and lateral phase-separation in lipid rafts, are believed to be vitally important for cellular recognition and fusion, signal transduction, receptor trafficking, and viral budding. In this talk, we will present results from two studies on phospholipid bilayers with small angle neutron scattering carried out at the Spallation Neutron Source (SNS) at Oak Ridge National Laboratory, peptide-induced lipid flip-flop in asymmetric liposomes, and melatonin-stabilized phase separation in phospholipid bilayer membranes. The results from these studies have important implications for understanding the roles that lateral and transmembrane compositional and structural complexity have for many biological functions.

D04.01.05
Characterization of Biomass-Degrading Enzymes Using Neutron Diffraction and Scattering
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Sensitivity to hydrogen/deuterium and lack of observable radiation damage make cold neutrons an

ideal probe for structural studies of redox enzymes. Neutron protein crystallography (NPC) is a powerful tool for investigating protein chemistry because it directly locates hydrogen atom positions in a protein structure [1, 2]. The visibility of hydrogen and deuterium atoms arises from the strong interaction of neutrons with the nuclei of these isotopes. Small-angle scattering (SAS) provides low resolution information on protein dimensions, described by the radius of gyration (*Rg*) and maximum dimension (*Dmax*). Combined with deuterium labeling and contrast variation techniques and modeling, small angle neutron scattering (SANS) further allows the structural investigation of individual components within protein-protein complexes. Fungal lytic polysaccharide monooxygenases (LPMOs) are copper containing metallo-enzymes involved in biomass oxidation. LPMO-catalyzed monooxygenation requires input of two electrons from LPMO redox partners, the cellobiose dehydrogenase enzymes (CDHs), and of one oxygen molecule to achieve hydroxylation of one carbon in the glycosidic bond [3]. The determination of the chemical nature of the oxygen species bound to the LPMO active site by X-ray crystallography is difficult. This is due in part to metal photo-reduction by exposure to the X-ray beam leading to a mix Cu(I)/Cu(II) catalytic center and to the lack of visibility of hydrogen atoms. In marked contrast to X-rays, neutrons do not cause radiation damage and are therefore well suited to study the enzymatic mechanism of redox enzymes. We will present our on-going X-ray and neutron crystallographic studies that provide new insight into the LPMO monooxygenation mechanism [4,5]. Redox complexes are often transient, and their structural characterization can be challenging. We are using SANS combined with the contrast match method to structurally characterize the intermolecular electron transfer between CDH and LPMO, in absence of X-ray induced photo-reduction [6].

References:
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D04.01.06
Ewald—A Macromolecular Diffractometer for the Second Target Station
Leighton Coates and Lee Robertson; Oak Ridge National Laboratory, United States

The Second Target Station (STS) of the Spallation Neutron Source (SNS) will provide unprecedented peak brightness of cold neutrons together with a broad bandwidth resulting from 15 Hz operation. These properties make the STS ideal for studying biological and soft matter systems with this in mind, an extended wide-angle Laue diffractometer, `Ewald,' has been designed STS. Ewald has been designed to rapidly collect data from crystals of macromolecules orders of magnitude smaller than is currently possible. This means that the development and construction of the STS will be a transformational event for macromolecular neutron diffraction studies. Visualizing hydrogen atoms in biological materials is one of the biggest remaining challenges in biophysical analysis. While X-ray techniques have an unrivaled capacity for high-throughput structure determination, neutron diffraction is uniquely sensitive to hydrogen atom positions in crystals of biological materials and can provide a complete picture of the atomic and electronic structures of biological macromolecules. This information can be essential in providing predictive understanding and engineering control of key biological processes, for example, in catalysis, ligand binding, and light-harvesting, and to guide bioengineering of enzymes and drug design. One very common and large capability gap for all neutron atomic resolution single-crystal diffractometers is the weak flux of available neutron beams, which results in limited signal-to-noise ratios giving a requirement for sample volumes of at least 0.1 mm in volume. The ability to operate on crystals an order of magnitude smaller will open up new and more complex systems to studies with neutrons, which will help in our understanding of enzyme mechanisms and enable us to improve drugs against multi-resistant bacteria.

Poster Session: Biology and Biotechnology

PD.01.01
Protein Interactions, Unfolding and Aggregation at Low Temperature and High Pressure
Jordan E. Berger[1], Susana Teixeira[2,1] and Christopher J. Roberts[1,2]; [1]University of Delaware, United States; [2]National Institute of Standards and Technology, United States

Aggregation of therapeutic proteins is a well-known issue that can occur during multiple steps of manufacturing and storage. Experimental models of aggregation under cold (sub-zero) storage conditions are often confounded because of the freezing transition for water and the presence of ice itself, which is also a potential denaturing agent. High pressure (HP) can be used to prevent water from crystallizing at sub-zero temperatures, allowing for the characterization of aggregate growth mechanisms and aggregate size/morphology. This work focuses on forced / accelerated degradation via high pressure (up to 350 MPa) and low temperature (0 to -20 °C), for a typical immunoglobulin IgG1 at common storage formulations. Intrinsic fluorescence and small-angle neutron scattering were used to observe the in situ effects of pressure and temperature in an effort to not only characterize behavior at these conditions, but also to delineate the effects of high pressure vs. low temperature on the kinetics and mechanisms of protein aggregation, including aggregation prone intermediates that are often difficult to characterize. Partial unfolding of the IgG1 monoclonal antibody (MAb) molecule was observed under a range of pressure/temperature conditions. Room temperature combined with high pressure resulted in irreversibly unfolded protein in terms of tertiary structure, while at subzero temperatures the data is consistent with reversible unfolding. Fourier Transform infrared spectroscopy (FTIR) was also used to monitor changes in secondary structure before and after incubation at elevated pressure and/or temperature. IgG1 retained native secondary structure after incubations, despite perturbed tertiary structure. Screening of pressure and temperature conditions was also used to monitor the respective contributions of the isolated MAb fragments (Fab and Fc fragments) to unfolding and aggregation of IgG1. At elevated pressure, the Fab fragment showed partial unfolding and significant aggregation at mild temperatures while the Fc unfolded to a larger extent. The results are consistent with reversible unfolding when the Fc is returned to atmospheric pressure. High pressure SANS will also be used to characterize protein-protein interactions under conditions that promote unfolding. The combined use of spectroscopic and scattering techniques provides insights into aggregation, interactions, and conformational stability of proteins that can ultimately lead to new stabilization strategies that guide faster development of safer MAb therapeutics.

PD.01.03

The Physics of Floating Lipid Bilayers

Dennis J. Michalak[1], Mathias Lösche[1] and David P. Hoogerheide[2]; [1]Carnegie Mellon University, United States; [2]National Institute of Standards and Technology, United States

Due to their ubiquity, versatility, and chemical diversity, lipid bilayers are an increasingly important component of engineered biosensors and biophysical measurement platforms. Bilayers supported on solid substrates are a common design feature of these systems, and the presence of the substrate introduces perturbations in the bilayer structure, affecting both stability and function. Thus, the ability to understand and finely tune the physical interaction between a bilayer and a substrate is essential. Here we show that the separation between a bilayer and its supporting substrate can be finely tuned at the 0.1 nm level, as measured by neutron reflectivity, by adjusting the surface charge of the substrate. Remarkably, the tuning is possible even with zwitterionic bilayers, which appear to carry a small but non-negligible surface charge. The combination of van der Waals and electrostatic forces therefore appears to dominate the bilayer-substrate interaction. The interaction free energy profiles derived from a DLVO-type theory with no free parameters quantitatively describes the experimental observations.

PD.01.04

SANS Investigation of the Nonstructural Protein (Nsp8) a Non-Canonical RNA Polymerase of SARS-CoV2

Wellington C. Leite[1], Qiu Zhang[1], Swati Pant[1], Yichong Fan[1], Jacob A. Sumner[1], Kevin Weiss[1], Mateusz Wilamowski[2], Robert P. Jedrzejczak[2], Andrezej Joachimiak[2], Shuo Qian[1], Sai Venkatesh Pingali[1] and Hugh O'Neill[1]; [1]Oak Ridge National Laboratory, United States; [2]Argonne National Laboratory, United States

A novel coronavirus (SARS-COV2) outbreak has caused a global pandemic resulting in hundreds of thousands of infections and thousands of deaths worldwide. The non-structural protein 8 (Nsp8) from SARS-CoV2 is a non-canonical RNA polymerase and plays an important role in the viral replication machinery. Nsp8 interacts with Nsp7, and previous *in vitro* studies showed that the RNA polymerase activity of Nsp8 is enhanced by Nsp7. In addition, Nsp8 has been shown to interact cooperatively with many other SARS-COV2 NSPs. In this work, we investigated the oligomerization state of Nsp7:Nsp8

complex in solution and its interaction with nucleic acids. Small-angle neutron scattering (SANS) intensity profiles were obtained in the Q-range from 0.008 $Å^{-1}$ < Q < 0.28 $Å^{-1}$. The radius of gyration (R_g) determined by the Guinier approximation is 32.0 ± 0.4 Å, and it did not vary over the measured concentration range (0.5 – 6.0 $mg.mL^{-1}$). The calculated molecular weight determined from the SANS experiments is 56 ± 3 kDa and 63 kDa from size exclusion chromatography. This indicates that Nsp7:Nsp8 has a dimer of dimers configuration in contrast to the crystal structure that showed a hollow cylindrical hexadecamer composed of eight copies of each of Nsp8 and Nsp7. The inner dimensions and electrostatic properties of this cylindrical structure is predicted to interact with nucleic acids. However, we did not observe such structure in solution, which suggests other mechanisms of protein-nucleic acid interaction. We then used the SANS with the contrast variation technique to investigate the interaction of Nsp7:Nsp8 complex with 23 'mer double stranded DNA (dsDNA) (Mw = 17 kDa). SANS was performed at the contrast match point of DNA (65% D_2O) to selectively highlight changes in the protein conformation. We observed that in the presence of the DNA substrate, the R_g of the Nsp7:Nsp8 DNA complex was 23.1 ± 1.5 Å, which agrees with the R_g of 25 Å derived from the crystal structure of an Nsp7:Nsp8 dimer. These results suggest that the Nsp7:Nsp8 oligomerization state is dynamic and can undergoes structural rearrangements as required during viral replication. Our results provide insight into SARS-CoV2 replication machinery that may be used to design novel therapeutic strategies for COVID-19.

PD.01.05

***Ab Initio* Structure Determination from Multiple Datasets Using SANS and SAXS—A DENSS Based Approach**

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Small-angle scattering, including SANS and SAXS, has long been a useful tool in structural biology to obtain biomolecular structures in solution, a more physiologically relevant condition. *Ab initio* methods from scattering data provide important structural information that agrees with other structural techniques. Joint *ab initio* structure determination from multiple datasets, such as complementary SANS and SAXS experiments and SANS contrast variation series, are desirable because they take advantage of additional structural information to improve reconstruction accuracy. SANS offers the unique capability of performing a contrast variation series with D_2O. A D_2O contrast variation series is useful for visualizing the structural arrangement of multi-component biological complexes where the components have different scattering length densities. This technique is typically done by varying H_2O:D_2O buffer composition to contrast match out particular components from the resultant scattering data as needed. Based on DENSS, an iterative structure-factor retrieval algorithm that performs *ab initio* reconstruction of electron densities from SAXS data, we have developed DENSS Multiple to concurrently reconstruct and merge density maps created for each SANS D_2O contrast provided to the program. Each SANS D_2O contrast reconstruction starts with the same random density, but the density is independently corrected based on the scattering length density of the H_2O:D_2O mixture for the respective contrast. Iterative merging is done both to preserve the 3D spatial alignment of the structure and to combine the contrast information into one coherent reconstruction. When the algorithm converges, the resulting structure can be used as a starting point for the DENSS reconstruction using complimentary SAXS data. This technique allows the SAXS data to 'refine' the SANS *ab initio* reconstruction into a model with greater detail. Similarly, this process can be inverted to refine an initial SAXS reconstruction with SANS contrast variation data to compare results. This approach can also reconstruct the low-resolution envelope and density map for SANS contrast variation data. Our method has been tested on single- and multi-phase systems from both simulated and experimental data and shown to be effective at reconstructing various systems, including lipid nanodiscs, protein complexes with deuterated components, and more. We hope to continue to develop this software to allow for straightforward, automatic distinction of individual phases to help further analyze and interpret multi-phase SANS data.