Posters

Posters: Protein Structure and Conformation III

1543-Pos
Mechanism of translocation by bacteriophage T7 helicase gp4 using AWSEM-Suite
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In the process of DNA replication, the efficient unwinding of the duplex parental DNA is mediated by DNA helicases. Helicases are chemo-mechanic motors that use the energy of ATP hydrolysis to translocate unidirectionally along with DNA. Helicase gp4 from bacteriophage T7 is a model system for studying helicase in DNA replication. However, how ATP hydrolysis drives the conformational changes and the unidirectional translocation remains elusive. Here, molecular dynamics simulations using coarse-grained protein model AWSEM-Suite and DNA model 3SPN2 were applied to explore the global energy landscape of the helicase gp4 translocation along ssDNA. We built the initial model based on recently solved lock-washer shaped homo-hexamer structures of gp4. The umbrella sampling was used to capture the large-scale conformational changes and analyze the free energy landscape and translocation. By comparing the simulations from ATP, ADP, and apo forms, we suggested that the translocation process can be broken to several rotation-guided steps. Furthermore, three intermediate states were identified during the progress that indicate the transition states. We also found the long helix that bridges the N-terminal and C-terminal domains of gp4 may play an essential role in the translocation. In summary, the AWSEM model can probe the translocation's conformational changes and provide insights into it.

1544-Pos
Topological analysis of high temperature enzyme activity
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In this work, we study the effects of temperature on the structure of Candida Antarctica Lipase B (CALB) to investigate its potential for its industrial applications owing to its activity even under extreme conditions. In a previous experiment, we showed very unusual results where resin-immobilized CALB used for esterification of siloxane polymers proceeded without bulk solvent, and whose rate increased with temperature in excess of 130*C. In this study we are looking for the topological and micro-structural properties that allows it to be active at temperatures that would destroy most other proteins, and comparing our results for protein conformational stability in different solvents to compare the state of active and inactive topologically and reveal the secret behind its unusual activity. Using topological data analysis, and applying a novel computational technique to the structure of CALB, we examined the topological features of the protein network of amino acids as a point cloud data in 3D space. To this end, we utilized persistent homology to study the topology-function relationship in two active and inactive states.From the persistent homology tool we propose to recognize the relationship between the topology-function of these macromolecules, and uncover how local modifications in the amino acid interaction network structure correlate to activity at the scale of the entire protein network.

1545-Pos
NMR illuminates the ligand and surface adsorption of autolysin-amidase from *Staphylococcus epidermidis*
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Bacterial biofilms on medical devices and implants pose a serious health challenge, and the interaction of bacterial surface proteins to both biotic and abiotic surfaces is an important first step towards bacterial colonization and biofilm development. The autolysin (AtlE) surface protein of *S. epidermidis* functions in cell wall homeostasis, cell separation and promote biofilm. The amidase catalytic domain (AmiE) of AtlE is a 26 kDa zinc-dependent peptidoglycan peptidase and is thought to play a role in bacterial surface attachment. Here, we have characterized the structure, function, and interaction of the AmiE with a serum-coated abiotic surfaces using isothermal titration calorimetry and solution NMR spectrometry.We find that the RDC-based solution structure of AmiE is similar to the previously determined crystal structure. The ^{15}N-relaxation rates (R_1, R_2, and hetNOE) indicate motions on various NMR time scales for the active site residues. The zinc binding to the AmiE domain is characterized by high affinity ($K_D \sim 30$ nM) and induces a local conformational change around the active site as determined by solution NMR. In contrast, the substrate (muramyl tripeptide) showed a much weaker affinity ($K_D \sim 60$ μM) and show line broadening effect of the active site residues upon binding. We also find that AmiE has propensity adhere to serum-coated surfaces, and significantly reduces staphylococcal biofilm growth on those surfaces. Moreover, surface interaction region of AmiE is away from the active site. These results reveal the structural and functional properties of the AmiE domain required for normal cell growth, and its role in surface adsorption and biofilm formation

1546-Pos
Optimization of a processing and analysis workflow for proteome-wide structural biology
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Fast photochemical oxidation of proteins (FPOP) is a valuable, mass spectrometry (MS)-based, biophysical technique used to probe protein structure, interactions, and conformational states. FPOP has recently been extended to the study of intact cells (IC-FPOP) and has been shown to modify thousands of proteins in a single experiment for proteome-wide structural biology. Although IC-FPOP can reveal critical structural information, the post experimental process requires a multistep sample workflow to prepare for MS analysis and a lengthy data processing workflow post-MS analysis. For these reasons, there are constraints on the amount of structural information that can be gained from IC-FPOP experiments. To address these limitations, we have incorporated a fully automated high throughput FPOP sample handling workflow via coupling the Biomek i5 sample preparation workstation with the Mini MS Sample Prep Kit from Thermo Scientific to provide an efficient and reproducible means of processing mammalian cells for MS analysis. By converting to automated sample handling, this portion of the workflow is faster and more reproducible than previous methods done manually. We have also generated an analysis package using the R programming language to process the MS data, calculate the extent of modification at the peptide and residue level, and generate graphs corresponding to the data. These analytical improvements robustly limit human error by reducing the number of steps that require manual manipulation and can analyze hundreds of proteins at the peptide- and residue-level in a fraction of the time of manual analysis. Through these advancements to the IC-FPOP workflow, we aim to improve the quality of data that can be produced and subsequently processed to obtain more structural information across the proteome.

1547-Pos
Altered protein dynamics and transition kinetics delineate the oncogenic potential in mutated kinases
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Kinases are a class of proteins that play essential roles in cell signaling, differentiation, and proliferation. They are frequently mutated in cancer and are the second-largest therapy target of specific inhibitors in clinical research. The activation status of mutated kinases in cancer can impact phenotypic outcomes not limited to tumor progression and drug sensitivity. Many approaches are implemented to quantify these phenotypic outcomes by controlling cell signaling through introducing mutations in kinase systems. These methods have been transformational by relying on specific gain-of-function mutations. To better understand this at the molecular level, the role of mutations in intrinsic kinase activity needs to be quantified. Free energy calculations obtained through enhanced sampling techniques of statistical mechanics have facilitated the understanding of structural stabilization of mutated kinases systems. However, quantifying the degree of alterations caused by mutated systems to protein dynamics and transition kinetics to infer the resulting relative severity between wild-type and mutated kinases is not well studied. We implement a computational suite combining enhanced sampling techniques of Metadynamics and INDUS to investigate the role of mutations in altering protein dynamics and transition kinetics. Additionally, our Boltzmann weighted correlation approach efficiently quantifies the alteration in protein dynamics obtained through the free energy landscapes sampling the transition in kinase systems from inactive to active kinase states. Moreover, the suite also investigates the long-timescale role of solvent water molecules in protein dynamics. Finally, we analyze our results with log P profiles obtained from Hydrogen Exchange experiments