2701-Pos Board B131
Understanding the Catalytic Mechanism of Laminaripentaose Producing β-1,3-Glucanase
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Laminaripentaose-producing β-1,3-glucanase (LPHase) catalyzes the hydrolysis of a long chain polysaccharide β-1,3-glucan into specific pentasaccharide oligomers. LPHase is a member of the glycoside hydrolase family 64 (GH-64) that play important roles during biomass degradation. Experimentally, the enzymatic mechanism of LPHase remains to be determined although it has been reported that Glu154 and Asp170 may serve as the active site residues of LPHase. Molecular modelling may offer more insight into the reaction pathway.
Several synthetic substrates of LPHase have been reported. We chose laminarihexaose as the substrate and docked one structure of laminarihexaose obtained from the Protein Data bank to the active site of LPHase. Molecular dynamics simulations using a combined quantum mechanical and molecular mechanical (QM/MM) method have been employed to study the hydrolysis of the glycosidic bond catalyzed by LPHase. Our results on the current LPHase - substrate complex suggest that the proton transfer from the catalytic general acid to the glycosidic oxygen be concerted with the nucleophilic attack at the anomeric carbon, and the free energy of activation is about 30 kcal/mol. Additional simulations on LPHase - substrate complexes are underway to characterize the LPHase catalyzed reaction pathway.

2702-Pos Board B132
X-Ray Structure of a Mammalian Stearoyl-Coa Desaturase-1
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Stearoyl-CoA desaturase (SCD) is conserved in all eukaryotes and introduces the first double bond into saturated fatty acyl CoAs. Since the monounsaturated products of SCD are key precursors of membrane phospholipids, cholesterol esters, and triglycerides, SCD is pivotal in fatty acid metabolism. Humans have two SCD homologs (SCD1 and SCD5), and mice have four (SCD1-SCD4). SCD1-deficient mice do not become obese or diabetic when fed a high-fat diet because of improved lipid metabolic profiles and insulin sensitivity. Thus, SCD1 is a promising pharmacological target in the treatment of obesity, diabetes, and other metabolic diseases. SCD1 is an integral membrane protein located in the endoplasmic reticulum, and catalyzes the formation of a *cis*-double bond between the 9^{th} and 10^{th} carbons of stearoyl- or palmitoyl-CoA. The reaction requires molecular oxygen, which is activated by a diiron center, and cytochrome b5, which reduces the diiron center. To better understand the structural basis of these characteristics of SCD function, we crystallized and solved the structure of mouse SCD1 bound to a stearoyl-CoA molecule at 2.6 Å resolution. The structure shows a novel fold comprising four transmembrane helices capped by a cytosolic domain. The acyl chain of the bound stearoyl- CoA is enclosed in a tunnel buried in the cytosolic domain, and the geometry of the tunnel suggests the structural basis for the regioselectivity and stereospecificity of the desaturation reaction. The structure reveals a dimetal center coordinated by a unique configuration of nine conserved histidine residues that implies a potentially novel mechanism for oxygen activation. The structure also illustrates a potential pathway for substrate access and product egress, and a possible route for electron transfer from cytochrome b5 to the diiron center.

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Probing and Manipulating Enzyme Activity and Conformational Dynamics by Single-Molecule Afm-FRET and Magnetic Tweezers-FRET Ultramicroscopy
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Single-molecule conformational manipulation provides unique methods for studying the relationship between function and structure of biomolecules, and for exploring novel properties of biomolecules under complex local environments. Enzymatic reactions are traditionally studied at the ensemble level, despite significant static and dynamic inhomogeneities. We have developed and applied AFM/Magnetic Tweezers force manipulation combined single-molecule spectroscopy to study the mechanisms and dynamics of enzymatic reactions involved with kinase and lysozyme proteins. Enzymatic reaction turnovers and the associated structure changes of individual protein molecules under pico-Newton force manipulations were observed simultaneously in real-time by single-molecule FRET detections. By a repetitive pulling-releasing manipulation of a Cy3-Cy5 dye labeled kinase molecules under the conditions with and without enzymatic substrates, we observed and analyzed the enzymatic conformational dynamics. We demonstrated that the enzyme conformational flexibility can be regulated by enzyme-substrate interaction, an experimental evidence of protein-substrate folding-binding interaction mechanism. Our new approach is applicable to a wide range of single-molecule AFM-FRET measurements for protein conformational changes under enzymatic reactions, including controls of enzymatic reactivity by mechanical-force manipulating protein conformations.
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2704-Pos Board B134
Moonprot: A Database for Proteins that are Known to Moonlight
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Moonlighting proteins comprise a class of multifunctional proteins in which a single polypeptide chain performs multiple biochemical functions that are not due to gene fusions, multiple RNA splice variants, or pleiotropic effects. The known moonlighting proteins perform a variety of diverse functions in many different cell types and species, and information about their structures and functions is scattered in many publications. We have constructed the manually curated, searchable, internet-based MoonProt Database (http://www.moonlightingproteins.org) with information about the over 200 proteins that have been experimentally verified to be moonlighting proteins. The availability of this organized information provides a more complete picture of what is currently known about moonlighting proteins. The database will also aid researchers in other fields, including determining the functions of genes identified in genome sequencing projects, interpreting data from proteomics projects, and annotating protein sequence and structural databases. In addition, information about the structures and functions of moonlighting proteins can be helpful in understanding how novel protein functional sites evolved on an ancient protein scaffold, which can also help in the design of proteins with novel functions.

Transcription

2705-Pos Board B135
Molecular Origins of Bimodal mRNA Copy Number Distribution
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Stochastic gene expression contributes to the variability of genetically identical cells in identical environments, giving rise to significant consequences in many biological processes. Generally, stochastic gene expression is explained by a two-state model of gene regulation, where a gene switches between on and off states. Transcription kinetics obtained from in vivo imaging is consistent with the two-state model. A theoretical model predicts that the distribution of mRNA copy numbers can be transitioned from a Poisson distribution to a bimodal distribution by modulating kon and koff. This transition is considered the origin of cell-to-cell variability. However, despite the versatility of the two-state model of gene regulation, little is known about the dynamics of the on-off switching and modulation. Many mechanisms of gene regulation have been proposed, including promoter binding of repressors and activators and, more recently, structural changes of DNA by a DNA binding protein.
In this study, we reconstructed prokaryote transcription and transcriptional regulation in vitro by using an E coli RNA polymerase (RNAP) and a λ phage promoter, and visualized mRNA production at the single molecule level by fast fluorescence in situ hybridization (fastFISH) to analyze the dynamics of mRNA production and its modulation.